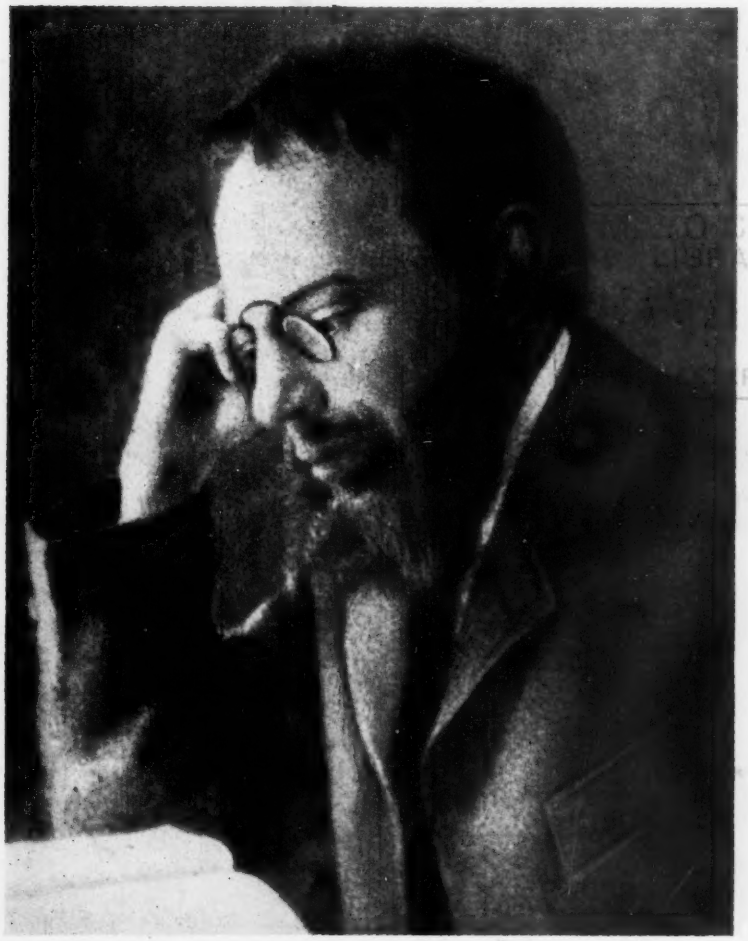
DIABETES

The Journal of the American Diabetes Association



L. V. SOBOLEV

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HYPODERMIC NEEDLES

DEVELOPED FOR ONE-TIME-USE

NEW SHARPER POINT

MEDICALLY TESTED PLASTIC HUB

A STERILE, NONPYROGENIC, NONTOXIC, B-D CONTROLLED NEEDLE

The Clinical Society of the New York Diabetes Association, an Affiliate of the American Diabetes Association, Fifth Annual Symposium Day on Diabetes Mellitus

Fat and Diabetes

The Fifth Annual All-Day Symposium of The Clinical Society of the New York Diabetes Association was held on Oct. 12, 1957, at Hunter College Playhouse Auditorium, New York City. Dr. Irving Graef, Chairman, presided.

CHAIRMAN GRAEF: May I introduce Dr. Alfred E. Fischer, President of the New York Diabetes Association, who would like to welcome you.

ALFRED E. FISCHER, M.D.: Today marks the Fifth Annual Symposium which has been sponsored by the New York Diabetes Association and its Clinical Society. This year the subject chosen is *Fat and Diabetes.*

A glance at the program will reveal that the speakers and their co-workers are, perhaps, the leading workers in the field of metabolism today.

It is particularly fortunate that a whole day can be spent having these investigators tell us the results of their latest endeavors.

I should like at this moment to express my personal thanks in advance to all of those who are participating in this Symposium for the time and the energy which they have spent in bringing their material before us.

I wish also to express our gratitude to Dr. C. J. O'Donovan and to The Upjohn Company, who have arranged to underwrite the cost of the Symposium. Without their help, this Symposium could not be held.

Ketogenesis

William C. Stadie, M.D., Philadelphia

The so-called ketone bodies are three in number: acetoacetic acid, β-hydroxybutyric acid, and acetone. The last, formed by the spontaneous nonenzymatic decarboxylation of acetoacetate, is, strictly speaking, the only true ketone. However, long usage has firmly fixed the term "ketone bodies" or "ketones" to designate these three substances. The precursors of the ketones are chiefly the long-chain fatty acids. Certain of the amino acids derived from protein are ketogenic; carbohydrates never yield ketone bodies. Ketogenesis is limited to the liver. The peripheral tissues do not produce ketone bodies from fatty acids. On the other hand, the kidney does form acetoacetate, but this is so rapidly oxidized by this organ that a net increase of ketone bodies does not occur. Ketogenesis occurs in the normal individual since traces of ketone bodies may be demonstrated in the blood and expired air. There are two conditions, however, in which ketogenesis is markedly increased, namely in starvation and in the diabetic animal.

The mode of formation of the ketone bodies from fatty acids in the liver is best understood by a brief review of the successive β-oxidation hypothesis originally developed

Presented at the Symposium on Fat and Diabetes sponsored by The Clinical Society of the New York Diabetes Association, Inc., on Oct. 12, 1957.

by Knoop.[1] A comparison of the present-day concepts with the older hypothesis clarifies the problem of ketogenesis. These concepts can be reduced to three: (1) Knoop demonstrated that long-chain fatty acids are oxidized at the β-carbons. (2) A two-carbon fragment, which was assumed to be acetic acid, was split off. The original fatty acid was now shorter by two carbons. (3) The β-oxidations were repeated until the original fatty acid molecule was reduced to a single molecule of acetoacetic acid, the remainder of the fatty acid molecule forming acetic acid. From these three concepts, three conclusions were implicit in the original formulation of the Knoop hypothesis. (1) One molecule of ketone body is produced per molecule of fatty acid dissimilated. (2) The two-carbon fragment presumed to be acetic acid, formed by successive β-oxidation, was utilized by the peripheral tissues. In the complete diabetic it became the chief source for energy production. (3) Acetoacetic acid, or its derivative, β-hydroxybutyric acid, is not further dissimilated by the liver or the peripheral tissues of the diabetic animal. In the conditions favoring ketogenesis, that is, starvation or diabetes, the production of the ketone bodies became excessive and led to acidosis.

Further experiments showed, one by one, that these conclusions were erroneous. A new version of the Knoop hypothesis, which is currently accepted, developed. First, it was shown that in diabetes essentially all of the fatty acid molecule oxidized in the liver was converted into acetoacetic acid. This evidence led McKay[2] to propose a modification of the original Knoop formulation. He postulated that the entire molecule of fatty acid was broken down by successive β-oxidation into two-carbon fragments. These two-carbon fragments were then condensed in a random fashion into the four-carbon acetoacetic acid. This concept of McKay was placed upon a sound experimental basis by the work of many investigators. Chief among them were Dr. Weinhouse and his collaborators.[3] They equilibrated liver slices with fatty acids containing isotopic carbon and isolated the ketone bodies which were formed. They demonstrated that the order of occurrence of the isotopic carbon in these was in accord with the hypothesis of McKay.

Second, attempts to demonstrate that the two-carbon fragment broken off by the oxidation of the long-chain fatty acids was acetic acid failed. For example, Stadie Zapp and Lukens,[4] using livers from depancreatized cats which were producing ketone bodies in large amounts, were unable to show even traces of acetic acid in such livers. They also failed to demonstrate the presence of intermediate fatty acids containing fourteen carbons or less. They concluded that once the oxidation of a fatty acid molecule was initiated, the reactions of successive β-oxidation were continued until the entire molecule was dissimilated, but acetic acid was not one of the products of oxidation. The long search for the elusive nature of the two-carbon compound ended with the discovery by Lipman of Coenzyme A, a compound containing pantothenic acid. Coenzyme A reacts so readily with the carboxyl group of the fatty acid that the two-carbon fragment split off by β-oxidation of the fatty acid never exists in the liver as acetic acid per se but as acetyl Co-A. For example, Lynen[5] showed that yeast respiring with alcohol or glucose in the presence of acetate formed acetyl Co-A. The work of these two authors and many others developed the current concept of the oxidative breakdown of fatty acids to acetyl Co-A and the reverse reactions of synthesis of the higher fatty acids from any compound yielding acetyl Co-A.

Third, the original version of the successive β-oxidation hypothesis of fatty acids postulated that in the diabetic the residual four-carbon ketone body was not utilized by the liver or peripheral tissues Subsequent experimental work by many investigators, however, demonstrated that both acetoacetate and β-hydroxybutyrate are freely utilized in the peripheral tissue. This was shown in perfused organs, eviscerated animals, and isolated tissue preparations from diabetic animals. Studies of the arteriovenous ketone difference in human diabetics also demonstrated this peripheral utilization of ketones.

In the diabetic cat, for example, Stadie, Zapp, and Lukens[6] measured the urinary output of ketone bodies and then sacrificed the animal and measured the ketone body formation by the liver slices in vitro. The data in

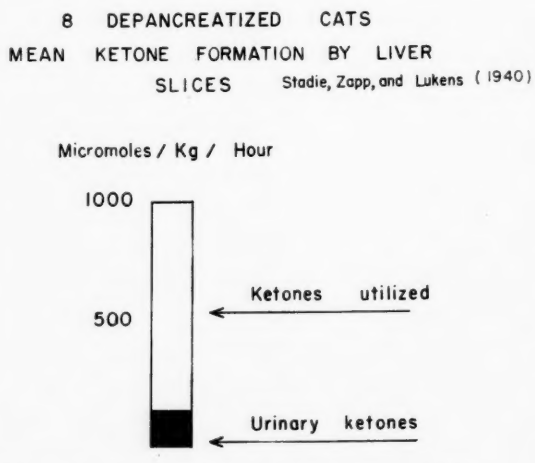

FIG. 1. Ketone formation by liver slices of diabetic cats.

FIGURE 2

Current concept of fatty acid oxidation

Reaction	Enzyme
(0) Activation $R\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COOH + ATP + CoA\text{-}SH \rightarrow$	Thiokinase $R\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CO\text{-}S\text{-}CoA + AMP + pyro\text{-}P$
(1) Oxidation $R\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CO\text{-}S\text{-}CoA + FAD \rightleftharpoons$	Acyl CoA dehydrogenase $R\text{-}CH_2\text{-}CH{=}CH\text{-}CO\text{-}S\text{-}CoA + FADH_2$
(2) Hydration $R\text{-}CH_2\text{-}CH{=}CH\text{-}CO\text{-}S\text{-}CoA + H_2O \rightleftharpoons$	Crotonase $R\text{-}CH_2\text{-}CHOH\text{-}CH_2\text{-}CO\text{-}S\text{-}CoA$
(3) Oxidation $R\text{-}CH_2CHOH\text{-}CH_2\text{-}CO\text{-}S\text{-}CoA + DPN \rightleftharpoons$	beta Hydroxyacyl CoA dehydrogenase $R\text{-}CH_2\text{-}CO\text{-}CH_2\text{-}CO\text{-}S\text{-}CoA + DPNH_2$
(4) Cleavage $R\text{-}CH_2\text{-}CO\text{-}CH_2\text{-}CO\text{-}S\text{-}CoA + CoA\text{-}SH \rightleftharpoons$	Thiolase $R\text{-}CH_2\text{-}CO\text{-}S\text{-}CoA + CH_3\text{-}CO\text{-}S\text{-}CoA$

figure 1 show that the urinary output was essentially zero while the ketone body formation was very great. The excess of ketone body production over ketone body excretion represents utilization by the peripheral tissues. The data emphasize another point which is often unappreciated, namely, that ketonuria is a very poor index of hepatic ketogenesis. In fact, ketonuria may be zero or low despite a very large production of ketones by the liver.

Subsequent work has shown that the peripheral tissues contain the necessary enzymatic system for the oxidation of ketone bodies. The reactions involved are: (1) Activation: acetoacetate plus succinyl Co-A produces acetoacetyl Co-A; (2) acetyl Co-A is then oxidized through the Krebs' cycle. Increased carbohydrate metabolism in the peripheral tissues is without influence upon the rate of utilization of the ketone bodies in the periphery, nor does insulin affect it.

ENZYMATIC REACTIONS INVOLVED IN THE SYNTHESIS AND OXIDATION OF HIGHER FATTY ACIDS

The enzymatic reactions involved in the synthesis and oxidation of higher fatty acids are shown in figure 2.

Following an initial reaction of activation, there are four reactions concerned with the oxidation of the higher fatty acids. (0) Activation. The reaction consists of the combination of acetyl Co-A through the sulfhydryl group to the carboxyl group of the fatty acid. The enzyme is the *activating* enzyme. (1) First oxidation. Two hydrogens, one each, are removed from the α and β-carbons with the production of an unsaturated fatty acid analogous to crotonic acid. The enzyme is acyl Co-A dehydrogenase and the co-factor is flavin adenine dinucleotide (FAD). (2) Hydration. Hydrogen and hydroxyl are introduced at the α and β-carbons respectively; a β-hydroxy acid is formed; the enzyme is crotonase. (3) Second oxidation. Two hydrogens are removed from the α and β-carbons of the β-hydroxy acid to form a keto acid; the enzyme is β-hydroxyacyl Co-A dehydrogenase and the co-factor is diphosphopyridine nucleotide (DPN). (4) Cleavage. Acetyl Co-A is split off and a fatty acid two carbons shorter than the original is formed. Simultaneously the shortened fatty acid combines with another molecule of Coenzyme A at the newly formed carboxyl group to yield an activated fatty acid. The enzyme is β-ketothiolase. This process is repeated until the entire fatty acid molecule has been reduced completely to acetyl Co-A. As already discussed, the acetyl Co-A molecules condense randomly in pairs with the formation of acetoacetyl Co-A. The Co-A is then split off by a deacylase with the formation of free acetoacetic acid. The enzymatic activity of deacylase is very high in the liver. In consequence, free acetoacetic acid is formed rapidly from the acetoacetyl Co-A and cannot be reactivated for further oxidation in the liver.

OXIDATION AND SYNTHESIS OF HIGHER FATTY ACIDS

On the basis of this analysis of the enzymatic systems concerned with the dissimilation of higher fatty acids in the liver, the diagram shown in the next figure is constructed. Oxidation of a higher fatty acid, such as palmitic with sixteen carbon atoms, yields eight molecules of acetyl Co-A. Two fates of the acetyl Co-A in the liver are of immediate interest: (1) oxidation through the Krebs cycle to CO_2, or (2) condensation to the ketone bodies followed by splitting off of the Coenzyme-A in conse-

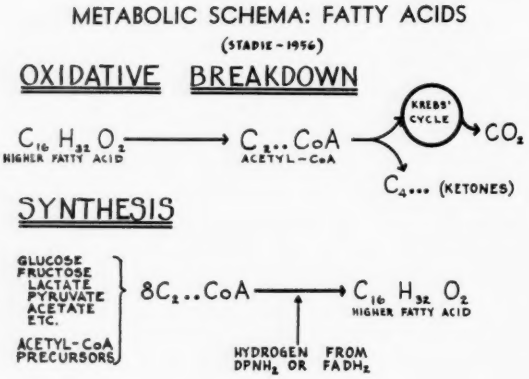

FIGURE 3

quence of which further oxidation of the acetoacetic acid in the liver ceases. Until a short time ago the processes of synthesis illustrated in the lower half of the figure were assumed to be the reverse of the reactions of oxidation. They were further assumed to be catalyzed by the same enzyme-coenzyme systems and to occur at the same intracellular locus. For fatty acid synthesis two requirements had to be met: (1) a precursor of acetyl Co-A is necessary. Some of these are listed in the figure. (2) A reductive environment considered to be derived from flavin adenine dinucleotide and reduced diphosphopyridine nucleotide. Condensations of two acetyl Co-A molecules in this reductive atmosphere led to the formation of butyryl Coenzyme-A. Further condensation led step by step to a building up of the molecule in the mammalian liver to a sixteen or eighteen-carbon fatty acid. When the fatty acids reached this length, deacylases which have been demonstrated to be present in the liver for these long-chain fatty acids split off the acetyl Co-A yielding the free acid.

FIGURE 4

Fatty acid metabolism of slices of liver from diabetic rats. (Data of Chaikoff et al.—1951)

Isotopic Nutrilites	Recovered istopic carbon: as CO_2	as higher fatty acids
Glucose	0	0
Fructose	Normal	0
Lactate	Normal	0
Pyruvate	Normal	0
Acetate	Normal	0

DIABETIC DEFECTS IN THE SYNTHESIS OF FATTY ACIDS

It has been known for a long time, originally from the work of Stetten and Boxer,[7] that fatty acid synthesis is impaired in the diabetic animal. This has been well substantiated by the work of many other investigators. I show here a summary of work done by Chaikoff.[8] He equilibrated liver slices from diabetic rats with media containing various metabolites uniformly labeled with isotopic carbon. With glucose as the metabolite, no radioactivity is found in the CO_2, a result fully to be expected and indicating the presence of the first metabolic block at an early stage of glucose metabolism. In addition, there is no fatty acid synthesis from the glucose.

With isotopic fructose the production of CO_2 is the same as that observed in the normal rat. This means that the formation of acetyl Co-A from fructose is unimpaired in the diabetic. But there is no incorporation of this acetyl Co-A into higher fatty acids. The same findings are observed using isotopic acetate. Except in the case of glucose, these data clearly mean that we have all the conditions necessary for fatty acid synthesis, namely, acetyl Co-A formation and, since CO_2 is formed, reduced cofactors as sources of hydrogen for purposes of reduction. Nevertheless, higher fatty acids are not synthesized. From the summation of this evidence, Chaikoff concluded that: "A second biochemical block must therefore be postulated for the diabetic liver, and the observations with C^{14}-acetate indicate that it lies in the path of conversion of a 'two-carbon-like' compound to fatty acids."

TWO PATHWAYS OF FATTY ACID METABOLISM IN THE LIVER

It appears from recent work that contrary to our previous conceptions, the reverse reactions of synthesis are not the same as those of oxidation of fatty acid.

FIGURE 5

	Acetate incorporated by cat liver slices into long chain fatty acids	Ketone formation by cat liver slices
	Brady, Lukens, and Gurin (1951) Micromoles/100 mg. Mean	Stadie, Zapp, and Lukens (1940) Micromoles/kg. cat Mean
Normal cats	2 - 8	240
Depancreatized cats	0 - 0.7	1,200
Houssay cats	5	85

Fatty acid synthesis from acetate and ketone formation by liver slices from normal, depancreatized and Houssay cats.

Consider these conclusions in relation to data obtained in studies on the metabolism of liver slices from depancreatized cats obtained in our laboratory and the laboratory of Gurin. Stadie, Zapp, and Lukens[6] studied the formation of ketone bodies by cat liver slices. Note the high rate of ketone formation (1,200) in the case of the depancreatized cat as compared to normal (240). In the depancreatized-hypophysectomized (Houssay) cat ketone formation is reduced to practically zero. Compare these data with the rate of incorporation by liver slices of acetate into long chain fatty acid observed by Brady, Lukens and Gurin.[9] In the depancreatized cat this incorporation is essentially zero but in the Houssay cat it is restored to normal.[5] Both sets of data can only lead to one conclusion, namely, that the enzyme systems causing the oxidative breakdown of higher fatty acids to acetyl Co-A from which the ketones are formed are unimpaired in the diabetic state; and second, that the enzyme systems catalyzing the synthesis of fatty acids from acetyl Co-A are impaired. This must mean that in the diabetic the two pathways, namely, oxidative breakdown, and the synthesis of fatty acids must differ in some important detail.

Further experimental evidence that there are two pathways of fatty acid metabolism, one of which is impaired in the diabetic, is indicated by the data shown in the next figure from experiments by Shaw from Gurin's laboratory.

FIGURE 6

Fatty acid synthesis by hepatic mitochondria from normal or diabetic rats
(Data of Shaw and Gurin—1956)

Added Co-factors: Mg; Citrate; DPN
Precursor of acetyl Co-A: Isotopic pyruvate

Source of mitochondria	Further additions	Fatty acid synthesis
Normal rat liver	Normal liver supernatant	Yes
	None	No
	Butyryl Co-A	Yes
Diabetic rat liver	Normal liver supernatant	Yes
	Diabetic liver supernatant	No
	Butyryl Co-A	Yes

Shaw, Dituri and Gurin[10] studied fatty acid synthesis by mitochondrial preparations made from livers of normal or diabetic rats. The system used consisted of four parts: (1) washed mitochondria either intact or lysed; (2) the supernatant from homogenized liver spun at high speed to remove all particulate matter; (3) certain cofactors such as magnesium, citrate, Coenzyme I (indicated as DPN) and (4) a precursor of acetyl Co-A, namely, isotopic pyruvate. The figure shows that in the case of mitochondria from normal rat liver, fatty acid synthesis from the isotopic pyruvate occurs when normal liver supernatant is included in the system. But synthetic butyryl Co-A alone gives the same result. Synthesis of higher fatty acids from the pyruvate then occurred at a rate comparable to that observed with normal liver supernatant. With mitochondrial preparations made from diabetic rat liver, the addition of supernatant prepared from the diabetic liver resulted in no fatty acid synthesis from pyruvate. With normal liver supernatant added, fatty acid synthesis did occur. The same, however, was observed with butyryl Co-A alone. On the basis of these experiments, Shaw and Gurin concluded that: "Butyryl Co-A appears to be able to replace the supernatant fraction of both the normal and diabetic water-soluble systems. It seems reasonable, therefore, to suggest that the diabetic liver is unable to convert pyruvate, acetate or acetyl Co-A to fatty acids, because it cannot convert acetyl Co-A to butyryl Co-A. This defect in the metabolism of the diabetic liver is presumably due to the inability of the soluble enzyme system to supply the co-factor or co-factors necessary for the reaction under discussion."

Recently Langdon[11] has published experiments which indicate that the inability of the liver from the diabetic animals to synthesize butyryl Co-A from acetyl Co-A may be due to the absence of reduced triphosphopyridine nucleotide. His evidence leads to the following conclusions: (1) the oxidation of the long chain fatty acids into acetyl Co-A proceeds by way of the two steps of oxidation already discussed, the first requiring flavin adenine dinucleotide for a co-factor, the second requiring diphosphopyridine nucleotide. Oxidation occurs within the mitochondria and not in the extramitochondrial portion of the liver cells. (2) The synthesis of fatty acids occurs only in the soluble enzyme systems contained in the extramitochondrial cytoplasm. The enzymatic systems in the mitochondria are not involved. The first reduction required for the conversion of acetoacetyl Co-A to β-hydroxybutyric acid requires, as in the case of oxidation, the co-factor diphosphopyridine nucleotide but in the reduced form ($DPNH_2$). (3) In contrast to the oxidation, however, the second step in reduction, namely the conversion of alpha-beta unsaturated acyl Co-A catalyzed by ethylene reductase requires reduced triphosphopyridine nucleotide ($TPNH_2$) as a co-factor rather than flavin adenine dinucleotide.

A summary of the data of Langdon from which these conclusions are drawn is shown in this figure. The data show that the incorporation of C^{14}-acetate into higher

FIGURE 7

Cell fractions active in fatty acid synthesis (Langdon—1957)

Mitochondria	Extramitochondrial cytoplasm	C^{14}-acetate incorp. mu moles
0	+	315
+	0	4

fatty acids is high when the enzymatic system is derived from the extramitochondrial cytoplasm. Contrariwise, in the presence of mitochondria alone, acetate incorporation is essentially zero. These new facts help explain the control of excessive ketogenesis in the diabetic animal when normal carbohydrate metabolism is restored by appropriate treatment.

FIGURE 8

Two sites for hepatic fatty acid synthesis

1 — $DPNH_2$ low in mitochondria = oxidative environment

2 — DPNH and TPNH high in extramitochondrial space = reductive environment

3 — Diabetic cat liver: ketogenesis high; fatty acid synthesis low

Figure 8 shows a summation of other evidence available in the literature indicating that there are two sites for enzymatic systems which are concerned with oxidation and the synthesis of fatty acid respectively. (1) The presence of a cytochrome system in the mitochondria makes the environment essentially an oxidative one. In consequence, reduced co-factors, such as diphosphopyridine nucleotide, are low. (2) On the other hand, in the extramitochondrial intracellular space the environment has a reductive character and the concentrations of reduced diphosphopyridine nucleotide and triphosphopyridine nucleotide are high. (3) And lastly, as we have already seen from the experiments of Gurin and of Stadie and their co-workers, in the diabetic cat liver slice ketogenesis representing oxidation is very high whereas fatty acid synthesis is low.

In figure 9, I show data taken from the literature showing the conditions which favor hepatic ketogenesis in the diabetic animal. (1) There is a diminished glucose uptake and (2) a low glucose oxidation. (3) There is an increased glucose-6-phosphatase. All these factors, of course, diminish the dissimilation of glucose. In consequence, the phosphohexose shunt, initiated by the oxidation of glucose-6-phosphate to phosphogluconate,

FIGURE 9

Conditions favoring hepatic ketogenesis in diabetic liver

1 — Diminished glucose uptake

2 — Low glucose oxidation

3 — Increased G-6-phosphatase

4 — Hexosemonophosphate path decreased

Thus: Decreased TPNH formation by liver

which is the chief source of reduced triphosphopyridine nucleotide in the liver, is decreased in activity. Further, it has been shown by Glock and McLean[12] that in the alloxanized rat there is a decrease of glucose-6-phosphate and phosphogluconic acid dehydrogenase, the enzymes which catalyze the first two oxidations of the phosphohexose shunt. All these factors make for a decreased $TPNH_2$ formation by the liver. Synthesis of fatty acids will fall and ketogenesis will tend to increase to maximum values. The oxidation of isocitric acid in the Krebs cycle is also linked with triphosphopyridine. A diminution of Krebs cycle activity owing to a diminution of glucose oxidation, will thus be another factor reducing the supply of $TPNH_2$ for the reductive reactions in fatty acid synthesis. All this may be summed up in the statement that fatty acid homeostasis in the liver, giving a balance of oxidation and synthesis of fatty acids, requires an adequate metabolism of glucose. In consequence, excessive ketone formation is avoided and ketosis does not occur.

I conclude with a final paragraph giving an explanation of the experiments of Stadie and Zapp and others, showing that once the initiation of oxidation of a long-chain fatty acid is initiated, the entire molecule of that particular fatty acid is completely dissimilated to acetyl Co-A. This appears to be explained by the type of deacylases which are found in the liver. Only two deacylases have been demonstrated, one for acetoacetyl Co-A and acetyl Co-A. No deacylase for higher fatty acids than carbon-four has been demonstrated. Once having been activated, a fatty acid molecule continues in an activated state until it reaches the four- or two-carbon stage. This explains the absence of fatty acids containing intermediate numbers of carbons in livers producing large amounts of ketone bodies from the higher fatty acids.

SUMMARIO IN INTERLINGUA

Cetogenese

Le conclusion de mi presentation es un paragrapho explicante como le experimentos de Stadie e Zapp e

alteros monstra que si tosto que le oxydation de un acido grasse a catena longe ha essite initiate, le integre molecula del acido grasso in question es completemente dissimilate a acetyl a coenzyma A. Isto pare esser explicabile per le typo de disacetylase que es trovate in le hepate. Solmente duo differente disacetylases ha essite demonstrate, un pro acetoacetyl a coenzyma A e un pro acetyl a coenzyma A. Nulle disacetylase pro acidos grasse a plus que 4 carbones ha essite demonstrate. Quando un molecula de acido grasse ha essite activate, illo continua in le stato activate usque illo arriva al stadio a 4 o a 2 carbones. Isto explica le absentia de acidos grasse con numeros intermediari de carbones in hepates que produce grande quantitates de corpores cetonic ab le acidos grasse superior.

ACKNOWLEDGMENT

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DISCUSSION

RICHARD J. CROSS, M.D.: I recall some twenty years ago hearing a lecture by one of the country's most brilliant biochemists, in which he summarized the state of knowledge of ketogenesis with the then popular cliché that fats burn in the fire of the carbohydrates, and, if the fire is not hot enough, they form smoke, and the smoke is the ketone bodies.

I think we have come a long way since then, as has been so ably summarized by Dr. Stadie. We have a distinct understanding of many of the steps which are involved. There are still many questions unanswered, but the pieces are beginning to fall into place, and it is always exciting when we can begin to correlate the observations of the biochemist in the laboratory with what the clinician has seen at the bedside.

The layman, perhaps, tends to judge science in terms of its practical and material accomplishments: the vial of vaccine or the Sputnik speeding overhead. The pure scientist supposedly is interested only in knowledge for its own sake and disdains this practical application. But we in the field of medical science tend to fall between these two extremes. We can appreciate the accomplishments of the basic scientist, but we also get a very real thrill when we begin to see these accomplishments tied in with the empirical observations of the clinician. And I think Dr. Stadie has indicated how this field is now approaching the point where these correlations can be made.

We should, however, use caution at exactly this point. Just because we are so anxious to make these correlations, we are in danger of being tempted too far out on a limb. There is many a slip between the Warburg cup and the bedside.

With reference, for example, to Dr. Stadie's indications that the supply of TPNH may be the factor that limits synthesis of fats and tends to cause the production of ketone bodies, this is a very interesting hypothesis and one which is indeed tempting. There are a number of factors we should consider, however, that lead us to adopt a cautious attitude with reference to this. For example, it is not even clear that these soluble enzyme systems which synthesize fatty acids are indeed the ones that supply the intact organism with fats.

I mention this because, despite numerous attempts in many different laboratories, so far as I am aware, nobody has as yet succeeded in making these enzyme systems produce anything approaching a quantitative yield of fatty acid from precursors. There is no question but that synthesis occurs. It can be clearly demonstrated with the use of isotopes, paper chromatography, etc. We know

that the road is there, but we are not at all clear that it is capable of carrying heavy traffic.

Furthermore, just because a biochemist can demonstrate that a particular factor, in this case TNPH, can be limiting in an in vitro experiment, he cannot assume that it is also a limiting factor under any circumstances in the intact organism. A liver cell contains a wide variety of substrates and co-factors of various sorts. Dozens of reactions are proceeding simultaneously, and new substrates and new co-factors are being formed and utilized all the time.

I personally find it a little difficult to believe that TPNH specifically becomes a limiting factor and would prefer to think of it in terms of a general lack of reduced pyridine nucleotides.

For these reasons, I think caution is indicated. On the other hand, I should not want these remarks to be interpreted as any sort of carping criticism of the ideas propounded by Dr. Stadie. It is only by setting up these working hypotheses that we can possibly make progress and that we can hope to unravel some of the mysteries that still becloud our understanding of ketogenesis.

A New Growth Factor

Several years ago it was found that the acetate requirement of certain micro-organisms could be replaced by natural materials which did not contain acetate. Subsequent work led to the isolation and characterization of lipoic acid which, in trace amounts, would replace the acetate requirement of some species of bacteria (*Nutrition Reviews 11*:59, 1953). This substance exerts a definite biochemical effect in bacteria and in animal tissues. Quite recently another acetate-replacing factor has been isolated and identified chemically, and it also appears to exert a specific biochemical effect in animal tissues.

H. R. Skaggs et al. (*J. Bact. 72*:519, 1956) observed that cultures of Lactobacillus acidophilus would not grow in their basal medium unless either sodium acetate or a sample of distillers' solubles was added. The activity of the distillers' solubles was much greater than could be accounted for by its acetate content. The basal medium contained lipoic acid, and all the known vitamins were found to be devoid of this acetate-replacing activity. Many natural materials were tested and found to exhibit activity in the assay. These included whole liver, liver extract, crude casein extract, whey and beer. It then appeared that some new factor, capable of replacing acetate for this particular organism, was present in these natural materials.

Steps were next taken to isolate this acetate-replacing factor in pure form. L. D. Wright et al. (*J. Am. Chem. Soc. 78*: 5273, 1956), starting with distillers' solubles, were able to isolate the factor in a fairly pure state.

The active material obtained from the isolation procedure was subjected to chemical studies (D. E. Wolf et al., *J. Am. Chem. Soc. 78*: 4499, 1956) and its structure was determined. The active compound was β-hydroxy-β-methyl-v-valerolactone. This compound was synthesized and found to be active in replacing acetate in the microbiologic assay, thus proving its structure.

Of particular interest are the observations relating β-hydroxy-β-methyl-v-valerolactone (DVA) to cholesterol synthesis (P. A. Tavorimina, M. H. Gibbs and J. W. Huff, *J. Am. Chem. Soc. 78*:4498, 1956). Rat liver homogenate was incubated with radioactive acetate, and cholesterol was subsequently isolated. As would be expected, the cholesterol contained radioactivity. The addition of unlabeled DVA depressed the incorporation of acetate into cholesterol. This suggested the possibility that the DVA was being utilized for cholesterol synthesis, thus replacing the acetate. In confirmation of this suggestion it was found that radioactive DVA was incorporated into cholesterol much more rapidly than was acetate. The magnitude of the utilization of DVA for cholesterol biosynthesis suggests that it may be the immediate precursor of cholesterol.

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Glycolytic Pathways

Their Relation to the Synthesis of Cholesterol and Fatty Acids

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Following the suggestion by Lipmann[1] and by Dickens[2] in 1936 that the glucose molecule can be broken down within the cell by a process other than Embden-Meyerhof glycolysis, an extensive amount of research has been carried out in an attempt to elucidate the details of this alternate route of glucose metabolism. Largely through the studies of Horecker[3] and of Racker[4] the reactions involved in the pathway, now known as the pentose phosphate route, or hexosemonophosphate shunt, have been very well described.

A summary of the more important of these reactions is shown in figure 1. Glucose-6-phosphate molecules, derived from glucose through the action of insulin, first lose one of their carbons as carbon dioxide to become five-carbon, pentose phosphates. Two of these pentose molecules then redistribute their total of ten carbons to yield one seven-carbon sugar, sedoheptulose phosphate, and one three-carbon sugar, glyceraldehyde phosphate. These ten carbons in turn can be converted to a four-carbon tetrose phosphate and a six-carbon fructose phosphate. Finally the tetrose phosphate can receive two carbons from another molecule of pentose phosphate to give a second fructose phosphate molecule plus one of glyceraldehyde phosphate. The net result of these reactions is that three molecules of glucose-6-phosphate have been converted into two molecules of fructose-6-phosphate, and one of glyceraldehyde phosphate, and in the process three carbons of the glucose molecule have been oxidized to carbon dioxide.

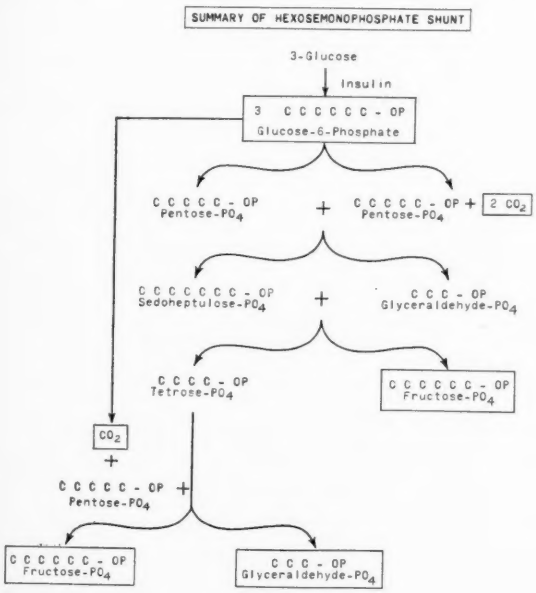

FIGURE 1

In contrast, when glucose is catabolized via the classical Embden-Meyerhof route, summarized briefly in figure 2, the glucose-6-phosphate is converted to phosphorylated fructoses from which *two* molecules of glyceraldehyde phosphate are formed. After further metabolism to pyruvate the carbons of the glucose molecule are oxidized to carbon dioxide, in part during the conversion of pyruvate to acetyl-CoA and the remainder in the Krebs' cycle. The points of departure and re-entry of the compounds of the hexosemonophosphate shunt are also shown in figure 2.

The relative importance of the hexosemonophosphate shunt and the Embden-Meyerhof routes in the breakdown of glucose has been the subject of numerous papers.[5,6,7,8,9] Unfortunately, no method of evaluating the quantitative significance of each of these pathways is entirely satisfactory;[10] however, the consensus of these studies is that in general the hexosemonophosphate shunt plays a relatively minor role in glucose metabolism accounting for less than 30 per cent of the glucose oxidized in the liver cell.*

Despite the relatively small part which is probably played by this glycolytic route in the process of glucose

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* It should be noted, however, that values for hexosemonophosphate shunt glycolysis approximating 50 per cent of the total glucose oxidized have been obtained by some investigators.[8,9]

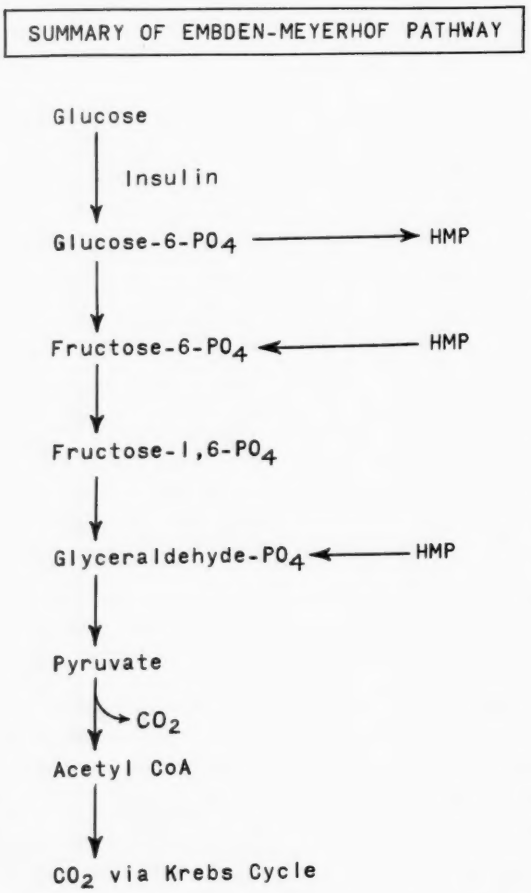

FIGURE 2

metabolism, we have recently been interested in studying the possible role of the hexosemonophosphate pathway in the regulation of other metabolic processes within the cell. Our initial investigations have been directed toward elucidating the influence of glycolysis on the synthesis of lipids.

It is now well established that one of the factors which controls the rate of fatty acid synthesis in the body is the amount of glucose metabolized per unit time. The feeding of excess glucose to normal animals is known to produce a marked stimulatory effect on the synthesis of fatty acids;[11,12] on the other hand, deprivation of glucose oxidation as seen in diabetes or in the fasting state results in a depression of fatty acid synthesis.[13,14] Accompanying these conditions ketone bodies accumulate, and the synthesis of cholesterol may be depressed;[15,16] however, especially in the case of diabetes the rate of cholesterolgenesis may be variable[15] and indeed at times has been found to be increased.[17] Re-establishment of glucose breakdown in either the diabetic or the fasted animal restores to normal the ability to synthesize both fatty acids and cholesterol, and ketosis is abolished; it is assumed, from this and other evidence, that in these metabolic states the defects in lipid synthesis are secondary to the lack of glycolysis.

Despite these observations, the mechanism by which glucose breakdown is able to exercise this striking influence on the metabolism of lipids has been poorly understood. We therefore began studies in normal[18,19] and diabetic[18,20] animals designed to determine whether one or the other of the two pathways of glucose metabolism might be primarily responsible for the *control* of lipid synthesis.

A means of approaching this problem was suggested by the fact, illustrated in figure 3, that the coenzyme, diphosphopyridine nucleotide (DPN), is required in

PATHWAYS OF GLYCOLYSIS IN LIVER

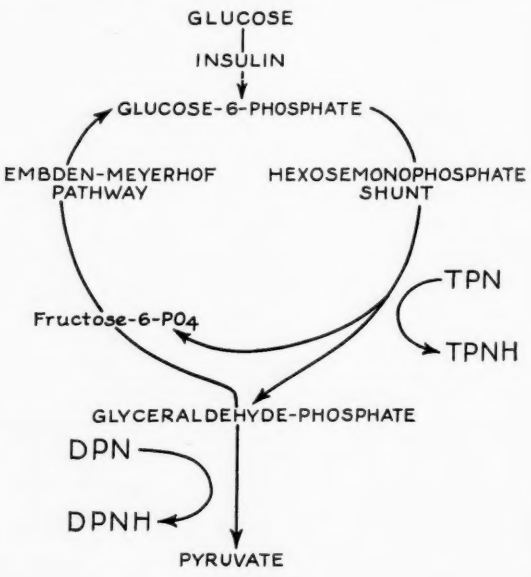

FIGURE 3

the operation of the Embden-Meyerhof pathway, while the coenzyme, triphosphopyridine nucleotide (TPN), is used by the hexosemonophosphate shunt. Wenner and associates[21] have indeed demonstrated that glycolysis can be increased by the addition of DPN to a liver homogenate and that under these circumstances the preponderance of glucose oxidation takes place via the Embden-Meyerhof pathway.[22] These authors have also presented evi-

dence that the addition of TPN to an homogenate, already supplemented with DPN, will increase the relative amounts of glucose oxidized by the hexosemonophosphate pathway. We have recently extended these studies and have shown that the addition of TPN alone to a liver homogenate will greatly increase the relative contribution of the hexosemonophosphate shunt to the process of glycolysis.[23]

An estimate of the relative contribution of each of the two routes of glycolysis can be obtained by studying the metabolism of glucose labeled with C^{14} on either its first or its sixth carbon. When glucose is oxidized by way of the hexosemonophosphate shunt, its first carbon is invariably lost as carbon dioxide. As a consequence, an increase in the relative amounts of $C^{14}O_2$ produced when glucose-1-C^{14} is used as the substrate of a metabolic system, indicates a shift in glucose metabolism toward the hexosemonophosphate pathway.

An example of such an experiment is shown in the first two columns of table 1. The addition of either DPN or TPN stimulates the oxidation of labeled glucose to carbon dioxide; however, it is apparent that DPN causes a more pronounced increase in the $C^{14}O_2$ derived from glucose-6-C^{14}, indicating a stimulation of the Embden-Meyerhof pathway, while TPN influences primarily the oxidation of the first carbon of glucose indicating an increased metabolism via the hexosemonophosphate shunt.

TABLE 1

Influence of DPN and TPN upon the routes of glycolysis

Cofactor added	$C^{14}O_2$ from		Per cent of fatty acid-C^{14} from	
	Glucose-6-C^{14}	Glucose-1-C^{14}	Embden-Meyerhof	HMP shunt
	cpm	cpm		
None	455	23,020	100	0
DPN	1,580	38,420	88	12
TPN	525	56,580	28	72
DPN & TPN	500	53,820	2	98

Cofactor concentrations: DPN: 0.7×10^{-3}M; TPN: 0.7×10^{-3}M; glucose: 71×10^{-3}M. All incubations in this and the following tables were carried out for 60 minutes at 37.5° C. under 95% O_2 — 5% CO_2.

A better approach to the quantitative evaluation of the two glycolytic pathways is to determine the per cent of fatty acid-C^{14} which is derived from each route of glycolysis.* Representative data,[23] given in the third and fourth columns of table 1, indicate that in the presence of DPN the preponderance of fatty acid-C^{14} comes by way of the Embden-Meyerhof route, while the addition of TPN shifts this ratio so that most of the lipid-carbon-14 is derived from the hexosemonophosphate shunt.

The addition of either TPN or DPN to an homogenate therefore provides a means by which glucose oxidation can be directed primarily down one glycolytic pathway or the other. By simultaneously determining the rates of cholesterol and fatty synthesis, the influence of each of these pathways upon the rates of lipid synthesis can be determined.

Such experiments have been performed in carefully prepared homogenates of normal[18,19] and diabetic[18,20] rat liver using the incorporation of acetate-1-C^{14} to indicate the rates of lipid synthesis. Glucose-6-phosphate, the first compound common to both pathways of glucose catabolism, was used as the substrate. Representative results from such studies on normal rat liver are shown in table 2.

TABLE 2

Effects of glycolytic pathways upon cholesterol and fatty acid synthesis in normal liver

Glycolytic pathway stimulated	Added acetate-C^{14} recovered in:		
	fatty acid	cholesterol	CO_2
	mu.Moles	mu.Moles $\times 10^{-2}$	mu.Moles
Neither	5	<3	270
HMP shunt	235	2,704	174
Embden-Meyerhof	5	5	276
EM + HMP	372	1,565	158
Neither	62	<5	853
HMP shunt	447	1,452	187
Embden-Meyerhof	26	<5	910
EM + HMP	447	716	359
Neither	10	<5	257
HMP shunt	723	228	60
Embden-Meyerhof	52	<5	252
EM + HMP	738	81	99

Concentration of additions: First experiment, glucose-6-phosphate 20×10^{-3}M, TPN and DPN 0.8×10^{-3}M, potassium acetate 2×10^{-3}M in a total volume of 2.5 ml.; second and third experiments, glucose-6-phosphate 18×10^{-3}M, TPN and DPN 0.7×10^{-3}M, potassium acetate 2×10^{-3}M in a total volume of 1.4 ml.

Stimulation of glycolysis via the Embden-Meyerhof pathway was found to cause only moderate and often no enhancement of fatty acid synthesis. On the other hand, stimulation of glycolysis over the hexosemonophosphate pathway consistently produced a very marked increase in fatty acid synthesis amounting in the experiments shown here to as much as seventy-two-fold. Stimulation of both routes of glycolysis usually, but not always, increased the yield of fatty acid-C^{14} still further.

We have concluded, therefore, that in normal liver

*This method was first suggested by Abraham, S., Hirsch, P. F., and Chaikoff, I. L.: The quantitative significance of glycolysis and nonglycolysis in glucose utilization by rat mammary gland. *J.B.C.* 211:31, 1954.

the synthesis of fatty acids may be primarily dependent upon the relatively small fraction of glucose which uses the hexosemonophosphate pathway.

The influence of glucose oxidation upon cholesterol synthesis is also shown in table 2. Again it is apparent that glycolysis via the Embden-Meyerhof route had little or no effect upon the rate of cholesterolgenesis, whereas stimulation of the hexosemonophosphate pathway increased cholesterol synthesis by at least 40- to 900-fold. It should be emphasized, however, that the stimulation of both pathways of glucose breakdown consistently depressed cholesterol synthesis relative to that caused by stimulating the hexosemonophosphate route alone, whereas the synthesis of fatty acids was usually further increased by such a procedure.

We have previously suggested from this data that when glucose is being rapidly metabolized, cholesterol synthesis may be controlled within the cell by the relative amounts of glucose which use each of the two glycolytic pathways; that which goes via the hexosemonophosphate route would increase cholesterolgenesis while that traversing the Embden-Meyerhof route would inhibit this process.[18,24]

The oxidation of acetate-1-C^{14} to carbon dioxide was usually stimulated relatively little by the addition of DPN or TPN to homogenates of normal liver. This would indicate that in these preparations, the pyridine nucleotides are probably present in optimal concentrations for the function of the Krebs cycle. Furthermore, this finding provides additional evidence that the observed stimulation of lipogenesis is not secondary to any influence of these added coenzymes on the tricarboxylic acid cycle.

As may be noted in figure 3 glucose catabolism via the Embden-Meyerhof pathway, while utilizing oxidized DPN, causes the production of DPNH, reduced diphosphopyridine nucleotide. DPNH can also be produced by the glucose oxidized over the hexosemonophosphate pathway if the glyceraldehyde-phosphate synthesized is then converted to pyruvate; however, the hexosemonophosphate shunt is the only route which can yield reduced triphosphopyridine nucleotide (TNPH). If the lipogenic influence of hexosemonophosphate glycolysis is mediated by a coenzyme, TPNH would seem therefore to be the most likely candidate to fill this role.

An attempt was consequently made to replace the hexosemonophosphate shunt in this preparation with another system capable of generating TPNH. Isocitrate and TPN in the presence of the appropriate enzyme will synthesize TPNH, and as noted in table 3 will stimulate fatty acid and cholesterol synthesis to approximately

TABLE 3

Influence of TPNH-generating systems upon fatty acid and cholesterol synthesis in normal liver

Cofactor and/or substrate	C-^{14}-acetate converted to: fatty acid	cholesterol
	mu.Moles	mu.Moles $\times 10^{-2}$
TPN + Potassium-isocitrate	314	550
TPN + Glucose-6-phosphate	133	960
TPN	17	190
Glucose-6-phosphate	4	5
Potassium-isocitrate	9	10

Concentration of additions: glucose-6-phosphate 21 $\times$ 10^{-3}M, potassium-isocitrate 21 $\times$ 10^{-3}M, TPN 0.8 $\times$ 10^{-3}M.

the same extent as will the hexosemonophosphate shunt. This experiment would indicate that in the normal liver it is probably the TPNH produced by the HMP pathway which is responsible for the influence of glycolysis upon both fatty acid and cholesterol synthesis. In this regard, Brady and associates[25] using the supernatant fraction of pigeon liver, and more recently, Langdon[26] studying liver homogenates from normal rats, have also shown that reduced TPN is a limiting factor in fatty acid synthesis.

As mentioned earlier, one of the characteristic metabolic lesions of the diabetic animal is a marked inability to synthesize fatty acids. Since Stetten and Boxer[27] first demonstrated this lesion, there has accumulated evidence to indicate that this "second block" in diabetic metabolism does not actually represent a second site of insulin action. On the contrary, as was first shown by Baker and associates,[28] the diabetic defect in lipogenesis seems clear-

TABLE 4

Effects of glycolytic pathways upon lipogenesis in diabetic liver

Glycolytic pathway stimulated	Added acetate-C^{14} recovered in: fatty acid	cholesterol	CO_2
	mu.Moles	mu.Moles $\times 10^{-2}$	mu.Moles
Neither	0.3	<3	8
HMP shunt	45	237	49
Embden-Meyerhof	4	<3	36
EM + HMP	58	168	54
Neither	0.7	<3	10
HMP shunt	85	49	38
Embden-Meyerhof	14	<3	27
EM + HMP	99	16	36
Neither	0.4	<3	9
HMP shunt	126	234	64
Embden-Meyerhof	5	<3	40
EM + HMP	55	26	28

Concentration of additions: glucose-6-phosphate 18 $\times$ 10^{-3}M, TPN and DPN 0.7 $\times$ 10^{-3}M, potassium acetate 2 $\times$ 10^{-3}M in a total volume of 1.4 ml.

ly to be secondary to the decreased ability of the diabetic animal to oxidize glucose. In view of this fact, it was decided to determine whether a deficiency of one or the other of the two pathways of glycolysis is primarily responsible for the lipogenic lesion of diabetes.

As is illustrated in table 4, the depressed level of fatty acid synthesis, which is readily demonstrable in diabetic liver homogenates, can be largely reversed by stimulation of glucose oxidation via the hexosemonophosphate route. On the other hand, an increase in glycolysis via the Embden-Meyerhof pathway has far less influence on this lesion.

In the typical experiments shown in table 4 stimulation of hexosemonophosphate glycolysis increased fatty acid synthesis about 100 to 300 times, whereas glycolysis via the Embden-Meyerhof route caused only a 10- to 20-fold increase in this process. Simultaneous stimulation of both of these pathways usually produced a further increase of fatty acid synthesis. Cholesterol synthesis in the diabetic liver was also enhanced by hexosemonophosphate oxidation but not by Embden-Meyerhof glycolysis.

The specific nature of the deficiency responsible for the inability of the diabetic to synthesize fatty acids was next investigated. As is illustrated in the data shown in table 5, an alternate source of TPNH, namely isocitrate and TPN, will also repair the diabetic lesions in fatty acid synthesis. Cholesterol synthesis is likewise stimulated by this source of TPNH.

TABLE 5

Influence of TPNH-generating systems on lipogenesis in diabetic liver

Cofactor and/or substrate	Acetate-C^{14} recovered in: fatty acid mu.Moles	cholesterol mu.Moles $\times 10^{-2}$
Glucose-6-phosphate	0.5	<5
TPN + Glucose-6-phosphate	40.6	99
TPN + isocitrate	71.2	36
TPN	10.9	73
Isocitrate	7.3	<5

Concentration of additions: glucose-6-phosphate 21×10^{-3}M, isocitrate 21×10^{-3}M, TPN 0.8×10^{-3}M.

We have concluded from these experiments, therefore, that the inability of the diabetic animal to synthesize fatty acids is due primarily to a lack of glucose oxidation via the hexosemonophosphate shunt, and that it is a deficiency of the TPNH normally produced by this pathway of glycolysis which is responsible for the characteristic diabetic lesion in fatty acid synthesis.

In order to understand the mechanisms by which the hexosemonophosphate shunt can influence lipogenesis, it is necessary to examine the biochemical steps leading to the synthesis of fatty acids and cholesterol from active acetate.

As illustrated in figure 4, the synthesis of both fatty acids and cholesterol begins with the condensation of two molecules of acetyl-coenzyme A (acetyl-CoA) to form acetoacetyl-CoA. This compound may lose its coenzyme A to become the ketone body, acetoacetic acid, or it may gain two hydrogens to yield β-hydroxybutyryl-CoA. β-hydroxybutyryl-CoA can in turn become the ketone body, β-hydroxybutyric acid or by reduction can be converted, after the addition of water and protons, to the activated fatty acid, butyryl-CoA. This compound can condense with successive molecules of acetyl-CoA to yield, eventually, long-chain fatty acids.

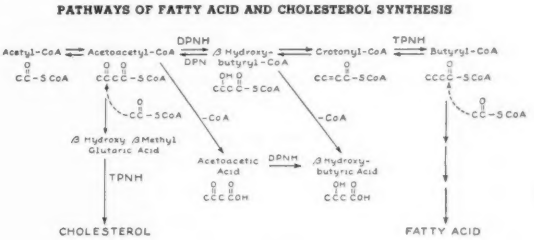

FIGURE 4

If, on the other hand, the condensation of acetyl-CoA occurs with acetoacetyl-CoA instead of with butyryl-CoA, the product formed is β-hydroxy-β-methylglutaric acid, a compound which is known to lead to the synthesis of cholesterol.[29]

Langdon[30] showed in 1955 that both DPNH and TPNH are required for the synthesis of fatty acids in liver. DPNH is believed to act at the reaction site, involving the reduction of acetoacetyl-CoA to β-hydroxybutyryl-CoA, while TPNH is required for the conversion of crotonyl-CoA to butyryl-CoA. Both Tchen and Bloch[31] and ourselves[32] have recently reported that TPNH is required

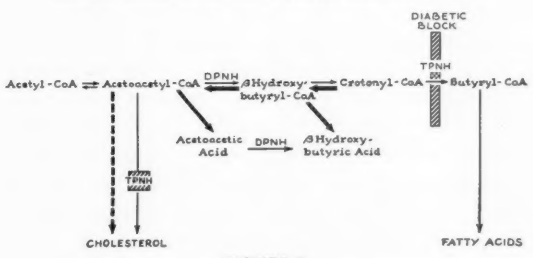

FIGURE 5

for the synthesis of cholesterol.

In view of these facts and of our finding that in diabetes the major block in fatty acid synthesis is due to a deficiency of TPNH, it would follow that the site of this second diabetic block is at the TPNH-dependent conversion of crotonyl-CoA to butyryl-CoA (see figure 5). The recent finding by Shaw and associates[33] that butyryl-CoA will stimulate the incorporation of pyruvate into fatty acids in diabetic liver had suggested that this lesion is located at some point prior to the formation of butyryl-CoA.

The location of the lipogenic lesion of diabetes at this site has an important bearing on several other metabolic abnormalities observed in clinical diabetes. Diabetic ketosis is characterized by the appearance of an excess of the ketone bodies, acetoacetic acid and β-hydroxybutyric acid. It would follow from our results that these two acids accumulate in part as a consequence of the lesion at the TPNH dependent step in fatty acid synthesis. Because of this metabolic block, coenzyme A derivatives of acetoacetic acid and β-hydroxybutyric acid would be prevented from participating in the further synthesis of fatty acids; as shown by the heavy arrows in figure 5, the concentration of these compounds would, as a consequence, rise; and their free acids would then accumulate either by the direct splitting off of coenzyme A or by the conversion of free acetoacetic acid to β-hydroxybutyric acid. There is no evidence, to my knowledge, that crotonyl-CoA or crotonic acid increases during diabetic acidosis, and this is probably due to the fact that equilibrium between β-hydroxybutyryl-CoA and crotonyl-CoA is known to favor the formation of the former compound.[34] In addition, our finding that reduced diphosphopyridine nucleotide, and hence the Embden-Meyerhof pathway of glycolysis, is a relatively unimportant limiting factor in the diabetic state is supported by the clinical finding that β-hydroxybutyric acid, the ketone body whose synthesis always requires DPNH, is present in large amounts in uncontrolled diabetes. It is apparent therefore that the classical chemical observations of clinical diabetes are consistent with our conclusions, based on in vitro studies, that a TPNH block is the important lesion in diabetic lipogenesis.

A decrease in the quantity of reduced triphosphopyridine nucleotide might be expected to depress cholesterol as well as fatty acid synthesis in diabetic liver since, as noted earlier, this cofactor appears to be specifically required in sterol synthesis. Such a depression in the synthesis of cholesterol has been reported;[15] however, cholesterol synthesis in diabetics has also been observed by some investigators to be above normal.[17] This latter finding may possibly be explained on the basis of an elevated concentration of acetoacetyl-CoA, which might at times accumulate to an extent sufficient to more than overcome the relative block caused by the decrease in TPNH.

In an analogous manner the relative depression in cholesterol synthesis produced in normal liver by the addition of Embden-Meyerhof glycolysis to the hexosemonophosphate shunt would be explained by an increase in fatty acid synthesis draining away either TPNH or acetoacetyl-CoA from the reactions leading to the synthesis of cholesterol.

The observation that a lack of TPNH is probably responsible for the derangements in fatty acid and cholesterol metabolism as well as for the ketosis of the diabetic state leads one to question whether other lesions of diabetes may be ascribed to a deficiency of this coenzyme. Some of the major biochemical lesions of diabetes are listed in table 6. The first three have already been discussed; a decrease in the level of the Krebs cycle intermediates has been demonstrated by Frohman;[35] and the decreased ability of the diabetic liver to synthesize proteins was shown in the experiments of Krahl.[36] In figure 6 are summarized some of the known sites of action of TPNH.

TABLE 6

Metabolic defects in diabetes

1	Fatty acid synthesis	Depressed
2	Ketone body synthesis	Increased
3	Cholesterol synthesis	Variable
4	Krebs cycle	Depressed
5	Protein synthesis	Depressed

The chief mechanism by which the four-carbon intermediates of the Krebs cycle are formed in the cell

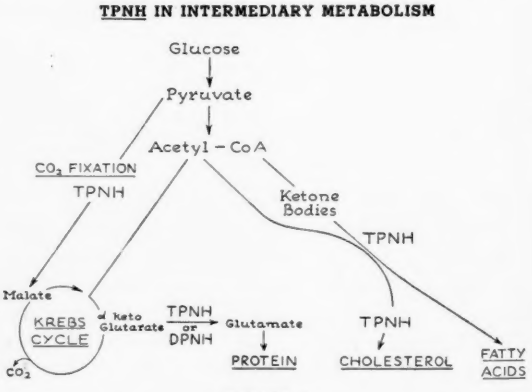

FIGURE 6

is now believed to be by the fixation of carbon dioxide to either pyruvate or phospho-enolpyruvate to yield respectively malate or oxalacetate. The former process, as shown in figure 6, is known to require TPNH as a cofactor.[37] It is reasonable to hypothesize therefore that the depressed level of four-carbon compounds in the Krebs cycle of the diabetic may be secondary to a lack of TPNH as well perhaps as to the frequently suggested decrease in pyruvate. Studies on this aspect of diabetic metabolism are now in progress in our laboratory.

Another known site of action of TPNH is in the conversion of α-ketoglutarate to the amino acid, glutamic acid. This is the one known process in the body which leads, either directly or indirectly, to a net synthesis of amino acids, and it is therefore not unlikely that protein synthesis itself may be dependent upon the rate of this reaction. Within the cell TPNH is probably the limiting cofactor in the synthesis of glutamic acid since, despite the fact that the glutamic dehydrogenase of the mitochondria can synthesize this amino acid using either DPNH or TPNH as the hydrogen donor,[38] the concentration of reduced diphosphopyridine nucleotide in the mitochondria is undoubtedly too small to allow DPNH to be an important cofactor in the reaction.[39] A deficiency of reduced TPN could therefore provide a theoretical explanation for the depressed protein synthesis which accompanies states of impaired glucose oxidation. Supporting this concept is the recent finding in our laboratory that the addition of TPN will cause a stimulation of protein synthesis in normal liver homogenates.[40]

SUMMARY

In conclusion, then, by use of liver homogenates of normal and diabetic rats we have attempted in these studies to examine the relationship between the two known pathways of glucose catabolism and the synthesis of lipids. It is concluded from these studies that in normal liver it is the glucose oxidized via the hexosemonophosphate shunt which may be primarily responsible for the effects of glycolysis in enhancing both fatty acid and cholesterol synthesis.

Evidence is presented to indicate that the cofactor mediating these effects of glycolysis is the coenzyme, reduced triphosphopyridine nucleotide.

Our data also suggest that cholesterol synthesis in the cell may be controlled by the relative amounts of glucose using each of the glycolytic pathways; hexosemonophosphate glycolysis stimulating and Embden-Meyerhof glycolysis tending to depress sterol synthesis.

The impaired synrhesis of fatty acids which is characteristic of diabetes is ascribed primarily to the deficiency in this disease of glycolysis via the hexosemonophosphate shunt; we believe that data referred to represent the first demonstration that the specific defect in diabetic lipogenesis is probably a lack of reduced triphosphopyridine nucleotide normally produced by this pathway.

An explanation of diabetic ketosis is presented based on a lesion in lipogenesis located at the site of action of reduced triphosphopyridine nucleotide; and finally, the possibility is examined that the metabolic lesions in the Krebs cycle and in protein synthesis observed in the diabetic state may also be due to a deficiency of the coenzyme, reduced triphosphopyridine nucleotide.

SUMMARIO IN INTERLINGUA

Vias De Glycolyse: Lor Relation Al Synthese De Cholesterol E De Acidos Grasse

Per le uso de homogenatos hepatic ab rattos normal e ab rattos diabetic nos ha examinate le relation inter le duo cognoscite vias del catabolismo de glucosa e del synthese de lipidos. Le examine supporta le conclusion que in un hepate normal, le oxydation de glucosa passante per le shunt de hexosomonophosphato es primarimente responsabile pro le effectos que le glycolyse produce in promover le synthese de acido grasse e de cholesterol.

Es presentate datos que indica que le cofactor que age como mediator in iste effectos del glycolyse es le coenzyma identificate como reducite triphosphopyridina-nucleotido.

Nostre datos etiam suggere que le synthese de cholesterol in le cellula es regulate per le quantitates relative de glucosa que usa cata un del vias glycolytic. Le glycolyse per le shunt de hexosomonophosphato stimula le synthese de sterol, e le glycolyse de Embden-Meyerhoff deprime lo.

Le defectiva synthese de acidos grasse que es characteristic de diabete es ascribite primarimente al deficientia (in iste morbo) del glycolyse que passa per le shunt de hexosomonophosphato. Nos crede que le datos hic citate representa le prime demonstration que le defecto specific del lipogenese diabetic es probabilemente un manco de reducite triphosphopyridina-nucleotido (que es normalmente producite per iste via).

Es presentate un explication de cetosis diabetic, stipulante como base un lesion del lipogenese al sito de action del reducite triphosphopyridina-nucleotido. Finalmente, es examinate le possibiltate que le lesiones metabolic in le cyclo de Krebs e in le synthese de proteina que es observate in diabete es etiam causate per un deficientia del coenzyma reducite triphosphopyridina-nucleotido.

ACKNOWLEDGMENTS

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An Abnormality of Nonesterified Fatty Acid Metabolism in Diabetes Mellitus

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Nonesterified fatty acids (NEFA) of plasma appear to be an important part of the transport system linking adipose tissue to liver and other working cells. With an exceptionally high turnover rate (half time in the neighborhood of two minutes) and responsiveness to hormones affecting carbohydrate metabolism, this fraction plays a role in the integration of carbohydrate and fat metabolism. Its precise role is still somewhat mysterious, but recent work has brought out a number of interesting facts.

Under conditions favoring synthesis of fat in adipose tissue—surplus of glucose, availability of insulin—the concentration of NEFA falls, while conditions leading to mobilization of fat from storage—fasting, or injection of epinephrine—cause a sharp rise in concentration. In the present work we asked whether the diabetic, like the normal subject, would show a close association between the state of his carbohydrate metabolism and concentration of NEFA. We were led by the hope that such an approach would help define the serious abnormalities of fat metabolism that occur in diabetes.

The NEFA level of normal or diabetic subjects in clinical control is markedly variable, even under standard, postabsorptive conditions. However, the diabetic group when compared statistically with the normals showed a significant elevation of mean NEFA concentration. Obese, nondiabetic patients also showed a greater than normal value. On the other hand, the blood glucose concentration of these various subjects showed no consistent correlation with NEFA level, presumably because a moderate elevation of blood glucose can be associated with an increase *or* a reduction of glucose utilization.

Associated with their abnormal glucose tolerance, the diabetic subjects showed an abnormally prolonged but comparatively weak fall of NEFA concentration after feeding of glucose or injection of glucagon. Patients in diabetic acidosis showed the most extreme changes: marked elevations of NEFA that correlated closely with the elevation of blood glucose, and sharp falls to normal in response to treatment with insulin.

The results suggest that elevation of NEFA may be of consequence in production of diabetic acidosis. The liver of the diabetic subject is known to have an impaired capacity for synthesis of fatty acids. Despite such a limitation most severe diabetics can be regulated fairly well, and only under the stress of infection or other acute disturbances become dangerously acidotic. We offer the hypothesis that the additional factor tending to precipitate acidosis in such subjects is failure in the control of NEFA release from adipose tissue, leading to a marked rise in plasma level, an overloading of liver with fatty acid substrate, and excessive production of ketone acids.

Presented at the Symposium on Fat and Diabetes sponsored by The Clinical Society of the New York Diabetes Association, Inc., on Oct. 12, 1957.

The abstract which is printed here was prepared by Dr. Vincent P. Dole. The full paper, of which he was co-author, was published under the same title in the November-December 1957 DIABETES, Volume 6, Number 6, and will be available in the Symposium reprints.

Group Discussion of the Two Preceding Papers

FORREST E. KENDALL, PH.D.: I suspect that the reason I was asked to discuss Dr. Siperstein's paper was that the Program Committee knows that, in spite of my profound ignorance of a subject, I am always willing to try to find some connection between the subject being discussed and the problem of atherosclerosis.

It is true that atherosclerosis is a problem of diabetes, causing, we are told, in this day of insulin treatment, at least half of the deaths occurring in diabetics.

Conversely, workers in the field of atherosclerosis have become convinced that atherosclerosis, like diabetes, is a disease of metabolic origin.

The difference between the two fields is that progress has been made in elucidating the abnormalities that give rise to diabetes. Very little progress has been made in pin-pointing the metabolic deficiencies that are believed to lie at the basis of atherosclerosis.

We feel the two fields must be intimately related. We have heard evidence presented this morning, connecting lipid metabolism with carbohydrate metabolism. They

are both part of the general metabolic process.

Can we, in the field of atherosclerosis, find any clues in the work presented this morning that will make our task easier? I think we can, particularly in the second paper, in which the connection between the work presented and atherosclerosis is more direct than the connection that can be found in the first one.

I think that the connection is this: The metabolic deficiency in lipid metabolism that causes atherosclerosis is unknown, but we believe that this deficiency is reflected in an increase in the beta-lipoprotein fraction of serum; in order to produce atherosclerotic lesions, the beta-lipoprotein level must be elevated above certain minimum values.

The increased beta-lipoprotein is not in itself a metabolic defect. It is produced by the defect. The normal process of fat metabolism seems to be that fat is mobilized from peripheral stores as NEFA's, nonesterified fatty acids, and carried to the tissues, where it is oxidized.

There is a possibility that the high levels of beta-lipoprotein found in human serum may be due to an inability of the individual to mobilize this fat as free fatty acid, and in face of this disability the individual is forced to mobilize it as beta-lipoprotein, giving rise to levels which, in combination with local tissue damage, leads to the development of atherosclerotic lesions.

Therefore, I think it would be of interest to know more about the parallelism, or the reciprocal relationship, between the NEFA's and the lipoproteins in the serum.

ROBERT S. GORDON, JR., M.D.: This is not the first time that I have had an opportunity to comment on a presentation by Dr. Dole or his co-workers at Rockefeller, and I hope it will not be the last. If any of you have heard me before, please forgive this very repetitive discussion. But once again I find that it is necessary to agree with virtually everything Dr. Dole says. Actually, today he has put me in a very difficult situation by including some of my own data in his discussion, so I have no possibility of disagreeing at all.

Still, there are two points that I should like to make that bear on the necessity of applying due caution in the interpretation of some of these results. First, is the difficulty of calculating total fat turnover in the liver on the basis of arteriovenous differences. Unfortunately, it is not possible in an intact human subject, which is our favorite experimental animal, to get samples of portal vein blood. Since the blood perfusing the liver is derived more from the portal vein than from the arterial system, and since portal vein blood drains an area which may be either removing nonesterified fatty acid from plasma or adding it, or both—and by this I refer to the gastrointestinal tract—these calculations must always be taken with some caution. The gastrointestinal tissues in a fasting organism appear to remove nonesterified fatty acids from the plasma, a conclusion based on some of our work with C^{14}-labeled nonesterified fatty acids in animals. However, since we have strong reason to believe that this material is added to plasma by adipose tissue, the mesentery, which also drains into the portal vein, could have a marked influence on the nonesterified fatty acid content of the plasma before it enters the portal vein and perfuses the liver. Nevertheless, the calculations must be correct in terms of order of magnitude, though they may be in error in detail.

The second point, which, again, may not be very significant if we explore it further, is that in our own Institute we have examined, in a rather limited number of normal subjects, the effect of Orinase on blood sugar and nonesterified fatty acid concentration. In our experiments, the dose of Orinase used was 1 gm., and it was given by mouth, so that the effect would be expected, perhaps, to be less dramatic. Nevertheless, this dose was adequate to produce in these subjects a considerable hypoglycemia, lasting for several hours. In a series of about six such studies there was no consistent change in circulating nonesterified fatty acid concentrations.

We are inclined to believe that since, in general, it appears that the utilization of carbohydrate is the significant factor in determining the fatty acid output of the adipose tissue, Orinase does not significantly enhance the utilization of glucose. However, this conclusion must be taken tentatively, because it is based on a rather small number of cases. Perhaps further examination of Orinase will resolve this minor discrepancy.

Having disagreed, as much as I can, I would now like to agree wholeheartedly with the main thesis of this presentation, which is that the circulating nonesterified fatty acid is a very important transport mechanism, and that it accounts for the removal of fatty acids from depots and their transport to the liver as well as to other tissues in which fatty acids are utilized. This mechanism is disturbed in diabetes mellitus, and the primary disturbance seems to me to be an exaggeration of the output of fatty acids from the depots into the plasma.

Since the appearance of the paper by Havel and Fredrickson, of our Institute, concerning the turnover of nonesterified fatty acid in the dog, Dr. Donald Fredrickson and I, with considerable help from two hardworking assistants, Miss Cherkes and Mr. Ono, have been exploring the results of administration of C^{14}-labeled nonesterified fatty acids into the venous circulation of intact

human subjects. Most of these subjects have been normals. By an analysis of the curve of specific activity of $C^{14}O_2$ expired by the subject as a function of time, and the curve of specific activity of circulating nonesterified fatty acid, we have been able to support the conclusion that the flux through this fraction is equivalent to 100 plus or minus an error factor of probably 20 or 30 per cent of the total fat metabolism of this fasting individual.

Table 1 is a bit of a side issue, but it again supports the idea that utilization of carbohydrate controls the production of nonesterified fatty acid and, hence, the blood level.

In one subject, a brittle, unstable juvenile diabetic who, at the time of the experiment, was without insulin, the infusion of glucose intravenously, at the rate of 1 gm. per minute for somewhat over three hours, had no appreciable effect in reducing the plasma nonesterified fatty acid concentration. "U.F.A." is my abbreviation for "unesterified fatty acid," which is the same fraction Dr. Dole refers to as nonesterified fatty acid; don't let this confuse you. Also, our figures are noted as milliequivalents, so that if multipled by 1,000, they will be comparable to the figures Dr. Dole presented.

On another day, with the experimental conditions as nearly identical as possible, we gave an equivalent infusion of fructose, with the result, which was fairly dramatic, that this sugar, in the absence of insulin, was effective, whereas glucose was not.

TABLE 1

Intravenous sugars in diabetes, 200 gm. infused in 200 min.

Time (min.)	U.F.A. mEq./L. glucose	fructose
0	0.99	1.39
60	1.12	1.56
100	1.11	0.94
140	1.26	0.71
170	1.34	0.50
200	1.12	0.29

Figure 1 bears on our $C^{14}O_2$ production experiments This is a conceptual scheme that we are working with (and I might say that Dr. Dole is working with a very similar one), indicating that, starting from the left-hand arrow, fatty acids enter the system by arriving in the plasma from the peripheral adipose tissue. This plasma nonesterified fatty acid component is very small, but is in very rapid equilibrium with a larger intracellular compartment, which, we believe, is lipid in nature but may or may not be in the free or nonesterified form. It may include such compounds as acyl-CoA. From this

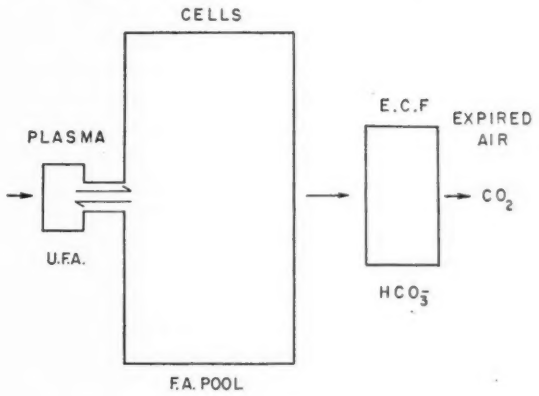


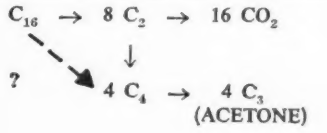
FIGURE 1

larger intracellular compartment, there is produced bicarbonate, which in turn gives rise to expired CO_2.

The injection of C^{14}-labeled unesterified fatty acid into a diabetic subject might be expected to give rise to radioactive acetone, since, as Dr. Stadie has explained, acetone is derived from the recombination of two-carbon fragments evolved in the degradation of fatty acids. If our current belief is correct, and the nonesterified fatty acids of blood plasma represent the means for the mobilization of fat, they should also function as the immediate metabolic precursors of the ketones. Let me refresh your memories by showing a reaction scheme by which palmitate might give rise to acetate and thence to ketones and carbon dioxide (table 2). The first step is the conversion of palmitate to eight acetates. The radiocarbon, in the carboxyl group of the palmitate, will label only the carboxyl carbon in the acetate so derived, and the molar specific activity will be one-eighth that of the palmitate. From this labeled acetate may be derived carbon-dioxide (with a radioactivity one-sixteenth that of the palmitate), or acetoacetate, and thence acetone. The decarboxylation of acetoacetate, whether occurring in the patient or in our processing of the urine, will selectively remove one of the labeled carbon atoms, so that the molar specific activity of acetone should be the same as that of acetate, and twice that of the carbon dioxide produced at the same time. Our calculations

TABLE 2

Tentative reaction scheme for acetone and CO_2 production from U.F.A.



may be slightly upset if acetoacetate bearing no radiocarbon can be derived from the last four carbons of the palmitic acid molecule without prior conversion to acetate, but this process, which is believed not to occur to any significant extent, could at worst introduce an error of about 25 per cent in our calculations of expected specific activity ratios.

Table 3 shows the observed molar specific activities of expired CO_2 and of urinary acetone when intravenous C^{14}-palmitate was given to a male diabetic who had been brought to a state of moderate acidosis by withholding insulin. The anticipated two-to-one ratio of specific activity is actually observed here. If we can believe our contention that plasma nonesterified fatty acid is the primary metabolic precursor of CO_2, it would appear that it must also be the precursor of acetone.

TABLE 3

CO_2 and acetone after C^{14}-U.F.A. i.v. in diabetic acidosis

Time (minutes)	Sp. Activity C/mM. Acetone	CO_2
0.10	0	103
10-20	40	202
20-30	296	225
30-40	354	210
40-50	528	206
50-60	441	200

(N.N. male, 31, juvenile D.M.)

I think this information should enhance the interest of clinical students of diabetes in the abnormalities in the behavior of this plasma nonesterified fatty acid. Perhaps we should suggest, in closing, that the abnormality of nonesterified fatty acid metabolism in diabetes is a very fundamental and a very significant one, and that, possibly, in the future measurements of nonesterified fatty acids may be useful in the clinic. Perhaps Dr. Dole's observation of higher nonesterified fatty acid concentrations in "controlled diabetics" indicates that they were not adequately and fully controlled.

GEORGE E. ANDERSON, M.D.: It is always of interest how truths tend to repeat themselves, and I am just harking back to a finding in 1930 of Professor A. B. McCallum of McGill University.

McCallum did some very careful studies on the quantitative aspects of tristearin released from fat stores, specifically changes in the beta-hydroxystearic acid content of the blood following definite dietary deficits in glucose in the normal individual on a fixed caloric intake. He found that, when there was a deficit of even 50 gm. (200 cal.), there was a release of stearins representing the release of some 228 gm. of the stearic acid representing fat amounting to approximately 2,000 calories, or ten times the deficit created.

In other words, apparently what Dr. Dole pointed out has been known and forgotten and is being brought up again—that a deficit in carbohydrate utilization very definitely produces a tremendous release of fatty acids from the fat stores of the body.

I think this is of interest in this situation.

PAUL A. MARKS, M.D.: In relation to Dr. Siperstein's very nice work, we have certain interesting observations on the red cells of patients in diabetic acidosis. We have been determining the levels of the activity of two enzymes which catalyze the oxidative steps of the pentose phosphate pathway, namely, glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase. These enzymes are involved in the generation of reduced triphosphopyridine nucleotide. In four of seven patients in severe acidosis, the level of activity of these enzymes was markedly reduced. Coincident with their clinical improvement and recovery from acidosis, the activity of these enzymes rose markedly. The order of magnitude was about two-fold. Frankly, we were very surprised at the magnitude of these changes. These observations are consistent with Dr. Siperstein's findings.

There is one other comment I would like to make with regard to the statement that the total amount of glucose going via the pentose phosphate pathway is small. I agree with Dr. Siperstein that there is no completely reliable technic for quantitating the relative importance of the alternative pathway of glucose utilization. However, the methods that have been based on determining the amount of CO_2 coming from either 1- or 6-C^{14}-labeled glucose, represent only a measurement of glucose that is oxidized to CO_2.

We have been attempting to evaluate the activity of this pathway with particular reference to the interconversion of glucose and ribose. In such experiments evidence is obtained for a very rapid equilibrium in vivo between ribose and glucose.* Our studies suggested that the extent to which these reactions contribute to glucose utilization may vary and is greatly influenced by the conditions of the study.

BENJAMIN JABLONS, M.D.: I should like to ask Dr. Siperstein a question.

In one of his slides, he showed that when TPN was

* Marks, P. A., and Feigelson, P.: Pathways of glycogen formation in liver and skeletal muscle in fed and fasted rats. J. Clin. Invest. 36:1279-84, 1957.

a factor, the level of cholesterol synthesized was, apparently, considerably higher than when DPN was a factor. But when he used a combination of the two, there was a considerable depreciation in the level of cholesterol synthesized.

Does the ratio of DPN to TPN exercise any influence in inhibiting the synthesis of cholesterol, or are these two factors entirely unrelated?

DR. SIPERSTEIN: In reply to Dr. Marks' very interesting comments, it is certainly true that the relative roles of the two glycolytic pathways have been found to vary considerably in the various normal and pathological states, and certainly the final answer to this problem, namely, how much goes down the hexosemonophosphate shunt, as we tried to emphasize, is still to be determined.

In answer to the last question, if I may, I think I might best answer that by showing again one of my figures (see figure 4, page 185).

I suspect that the explanation for the depression in cholesterol synthesis when we stimulate both pathways of glycolysis, is essentially the converse of the explanation I gave for the elevation in cholesterol synthesis noted in diabetes by some observers.

We would suggest that when we add Embden-Meyerhof glycolysis to hexosemonophosphate glycolysis, we are further stimulating fatty acid synthesis to the point where we are limiting either the TPNH or the acetoacetyl-CoA, which is available for cholesterol synthesis, and in this way, perhaps, Embden-Meyerhof glycolysis is able to cause increased fatty acid synthesis, so that it robs cholesterol synthesis of necessary cofactors or substrate.

DR. DOLE: I would only like to thank Dr. Gordon for his usual gracious remarks. As he came off the platform, I whispered to him—and I might as well confess this to the public—that he had so much new to say today, I am sorry he did not give the paper and let me comment on it.

Essential Fatty Acids and Human Nutrition

There has been a widespread feeling that essential fatty acids are not as important in human nutrition as they are in animal nutrition. Such a conclusion does not seem to be warranted in the face of present information. First, the general metabolic patterns are the same in animals and in humans, and one should not expect an exception in the case of the essential fatty acids. The inability to demonstrate dermal abnormalities in adult humans fed low-fat diets is inconclusive, because the duration of the attempts was too short, the diets were not rigidly free of essential fatty acids, and an adult human has more than a pound of linoleic acid in his body. Moreover, essential fatty acid deficiency has been induced in adult animals only once, and then by very drastic conditions compared to those used to induce deficiency in weanling animals. On the other hand, Hansen and Wiese (*Fed. Proc.* 16:387, 1957) have demonstrated conclusively that essential fatty acids are required by the infant, for the skin lesions induced by feeding low-fat infant formulas responded to oral administration of linoleic acid. Although dermatitis has not been induced in adult humans by fat-free diets, changes in the dietary fat cause alterations in plasma lipids. For example, the feeding of a diet containing only saturated fat increases the ratio of trienoic to tetraenoic acids in human plasma (*Proc. Soc. Exp. Biol. & Med.* 96:705, 1957). This parallels the observations that the tissues of animals deficient in essential fatty acids contain more trienoic acid than do normal tissues. These evidences suggest strongly that essential fatty acids are indeed required by humans.

Linoleic and linolenic acids, which are the precursors of the more highly unsaturated acids in animal tissues, are found in abundance in the common vegetable oils. The more highly unsaturated acids are found only in animal tissues. In muscle meats, the content of these is low compared to that of the nonessential fatty acids, but the fats of glandular tissues contain a much higher content of these acids. The fish oils also contain a high proportion of polyunsaturated acids. A diet containing a variety of vegetables, grains and meats should provide adequate quantities of essential and polyunsaturated acids. However, if it is true that essential fatty acid requirements are proportional to the intake of nonessential fatty acids, it would be wise to add to the concept of the balanced diet a consideration of the balance between essential and nonessential fatty acids in the dietary fat.

Ralph T. Holman, Ph.D., in *Nutrition Reviews*, 16:2, p. 35

Serum Triglycerides* in Health and Diabetes

Margaret J. Albrink, M.D., and Evelyn B. Man, Ph.D., New Haven

The historical observation that individuals with uncontrolled diabetes may have high concentrations of serum lipids and may also have an increased incidence of arteriosclerosis has much to do with the present-day theories relating serum lipids and arteriosclerosis. The fact that the lipemia of diabetes is related to defective carbohydrate metabolism rather than to fat in the diet is often overlooked in incrimination of dietary fat. This Symposium offers an opportunity to discuss factors other than dietary fat which may influence serum lipids. The purpose of this report is to describe variations in serum lipids occurring with fluctuations of carbohydrate metabolism and to focus attention on the significance of serum triglycerides in relation to other serum lipids.

Before the insulin era, lactescence of blood due to accumulation of fat in plasma was reported for certain diabetic patients. While this was thought to be a bad prognostic sign, its relationship to the metabolism of carbohydrate could not be evaluated until the advent of insulin. In 1934 Man and Peters,[1] using accurate quantitative methods, observed the precipitous descent often in twenty-four hours of serum lipids of patients in diabetic acidosis. Initial high concentrations of lipids dropped to normal or even low concentrations in response to administration of insulin, glucose and fluids. As insulin came into general use the serum lipids of controlled diabetics were usually in the normal range, regardless of the amount of dietary fat ingested.[2,3] In the early days of insulin when high fat diets were still in vogue it was not uncommon to find normal serum lipids even when the diet contained as much as 200 gm. of fat daily.

Presented at the Symposium on Fat and Diabetes sponsored by The Clinical Society of the New York Diabetes Association, Inc., on Oct. 12, 1957.

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* The term "triglycerides" is used in preference to the term "neutral fat." By our methods the triglyceride fatty acids represent the difference between total fatty acids and the sum of fatty acids esterified with cholesterol or combined in phospholipid molecules.

To explore further the relationship between serum lipids and carbohydrate metabolism the chief categories of serum lipids and their interrelationship are here reviewed. With the exception of small but highly significant amounts of fatty acids in the nonesterified form, fatty acids are transported in serum as esters in three major lipid compounds: cholesterol esters, phospholipids, and triglycerides.[4,6] By hydrolyzing the lipids the total fatty acids thus liberated can be titrated and calculated as milliequivalents of acid. By determining also the free and esterified cholesterol and the fatty acids of phospholipids from measurement of lipid phosphorus, the triglycerides can be estimated by difference between total fatty acids and the fatty acids combined in cholesterol esters and phospholipids.[5]

Table 1 shows the distribution of total fatty acids in terms of milliequivalents per liter in a controlled, in an uncontrolled, and in an acidotic diabetic. In the controlled diabetic, both the serum cholesterol of 215 mg. per cent and the triglycerides of 3.3 mEq. are normal. In the poorly or not controlled diabetic, although the cholesterol of 175 is normal, both total fatty acids of 19.3 and the triglyceride fatty acids of 10.0 mEq. are about 4 mEq. above the concentrations of these constituents in the sera of normal adults.[5,6]

The diabetic in acidosis[7] had a cholesterol of only 322 mg. per cent, about one and a half times the normal average, but triglyceride fatty acids of 38.6 mEq., ten times the normal average for triglycerides. That triglycerides are the first lipid fraction to be affected by lack of control of diabetes was also illustrated by the data of Chaikoff, Smith and Gibbs in their study of diabetic children.[8] Serum lipids were normal when diabetes was well regulated, but with lack of regulation, fatty acids varied more than other lipid fractions and the increases in fatty acids were not accompanied by abnormal concentrations of cholesterol. This illustrates the fact that determinations of cholesterol alone may not reflect a true image of the amounts of the other lipid fractions in the serum.[4,6,9,10]

In previous reports from this laboratory, the distortion of hyperlipemia in diabetic acidosis has been illustrated by data showing that triglycerides were most affected, cholesterol least influenced, and that phospholipids occu-

TABLE 1

Serum triglycerides and lipid fractions in controlled and uncontrolled diabetes

Diabetic status	Blood sugar mg. per cent	Total cholesterol mg. per cent	Lipid phosphorus mg. per cent	Fatty acids, mEq./L.						
				Total		Cholesterol esters		Phospholipid		Triglyceride
Controlled		215	9.7	12.9	—	(4.0	+	5.6)	=	3.3
Not controlled		175	10.7	19.3	—	(3.1	+	6.2)	=	10.0
Acidosis										
Admission	495	322	26.4	58.8	—	(4.9	+	15.3)	=	38.6
9 hrs. later		185	11.1	20.5	—	(3.1	+	6.4)	=	11.0
19 hrs. later	114	189	12.0	19.5	—	(3.1	+	7.0)	=	9.4
115 hrs. later	70	170	10.9	17.9	—	(2.9	+	6.3)	=	8.7

pied a middle position.[1,7] The height of the hyperlipemia was related to the ketosis and was in excess of the effects of hemoconcentration which accompanied dehydration. In severe ketosis synthesis from acetyl Co-A of long-chain fatty acids is impaired, and the effect of accumulation of acetate fragments on an increase in rate of synthesis of cholesterol has been suggested.[10,11,12] Synthesis of cholesterol from labeled acetate can be a rapid metabolic procedure. The speed with which labeled acetate is incorporated in cholesterol has been reported.[13]

In those first nine hours of treatment of diabetic acidosis the serum triglycerides fell to the baseline value for the patient although the initial elevation of triglycerides was much greater than the increases of the other lipid fractions. Frequently during treatment of typical diabetic acidosis the elevation of triglycerides exceeds the initial concentration of 38.6 mEq. shown in table 1 and the diminution in lipids sometimes requires twenty-four hours of treatment—a comparatively short time considering the magnitude of the hyperlipemia before treatment.[1,7] Nonesterified fatty acids which are elevated in diabetic acidosis[14] and which are removed within an interval of only a few minutes may play a major role in the decrease of triglycerides.[15,16,17]

Although the concentration of serum triglycerides is indicative of control of diabetes, triglycerides, because there has been no satisfactory method for their direct determination, have been somewhat neglected. They have a major influence on the concentration and physical status of the other serum lipid components.[4,9] In special instances elevation of triglyceride fatty acids in diabetic patients is the result of renal disease or primary lipoidosis. Triglycerides, previously called neutral fat, are the chief lipid components of dietary fats and oils, and of adipose tissue. Ingested triglycerides, absorbed from the intestinal lacteals into the thoracic duct[18,19] and thus delivered to the circulation, are the chief lipid component of chyle and chylomicra.[20] A temporary rise in concentration of triglycerides occurs after a fat meal and is associated with the lactescent appearance of serum, but this transient, meager increase of only about 3 mEq. per liter[21] decreases after a few hours. Normal postabsorptive serum is clear, the lipids existing as water soluble lipid-protein complexes, the lipoproteins. However, the ability of serum proteins to hold triglycerides in solution is limited. If the disposal of chylomicra from blood after a fat meal is inefficient, triglycerides increase progressively. Permanent lactescence, persisting into the postabsorptive state, results if triglycerides exceed a concentration of about 20 mEq./L.[22] These minute fat particles rise to the top of serum after relatively gentle ultracentrifugation at 18,000 RPM for one hour, leaving the water soluble lipids undisturbed in the clear infranate. This speed is much too slow to influence significantly the lipids present in clear serum even though the concentration of cholesterol and phospholipids may be high.[22]

In figure 1 are shown the concentrations of triglycerides in the "cream" layer removed by ultracentrifugation, and in the remaining subnatant fluid, in sera of varying concentrations of triglycerides. An increase in triglycerides of the subnatant layer reaches a limit at about 20 mEq./L. All further increases are measurable not in the subnatant but in the "cream" layer. The appearance of serum makes such increases evident even before centrifugation.

Persistent lactescence of serum, resulting from accumulation of triglycerides, is an unusual pathological state occuring only in a few diseases, of which diabetic acidosis is one of metabolic interest. The source of such triglycerides is not clearly understood. They are probably derived in part from the diet, but also from endogenous sources. An increased concentration of triglycerides in serum is the prime prerequisite for the development of lactescence but in abnormal states, the lipid particles which cause the lactescence and are removed by gentle centrifugation, consist not only of triglycerides, but also of cholesterol and phospholipids in proportions commensurate with the concentration of triglycerides. An extreme

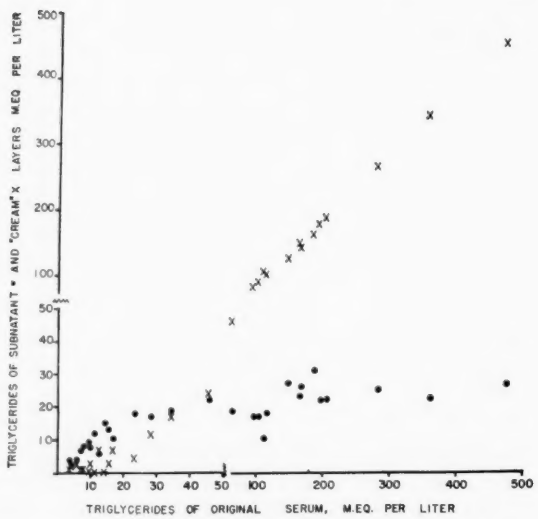

FIG. 1. Triglyceride fatty acids of original serum and of subnatant and cream layers, after centrifugation. From Albrink, M. J., Man, E. B., and Peters, J. P.: J. Clin. Invest. 34:147, 1955.[22]

case may illustrate, in exaggerated form, the possible influence of triglycerides on the other lipid components. Figure 2 shows the distribution of cholesterol between the "cream" and subnatant layers of successive sera obtained from a diabetic patient with extreme elevation of triglycerides and lactescence of his serum. In the initial serum when the triglycerides approached 500 mEq./L., 80 to 90 per cent of the cholesterol was found in the "cream" layer and the cholesterol in the subnatant layer was low. As the lactescence cleared progressively more of the cholesterol was found in this clear layer, and the total cholesterol gradually returned to normal.[22]

Similar alterations in the flotation properties of cholesterol can be produced if triglycerides are increased by the rapid intravenous infusion of fat. Three malnourished patients received intravenous fat emulsions* because of malnutrition. Sera were studied before and after the infusion of 90 gm. of fat. Before the infusion, cholesterol and phospholipids were present in normal concentrations, and not more than 5 per cent was removed by centrifugation. At the end of the infusion the serum was extremely lactescent and from 30 to 50 per cent of the cholesterol was now removed with the cream while the cholesterol of the subnatant fluid was correspondingly reduced as is shown in figure 3. In these instances the total cholesterol increased, al-

*"Lipomul I.V." was kindly supplied by Dr. Edward A. Hawk of The Upjohn Company, Kalamazoo, Michigan.

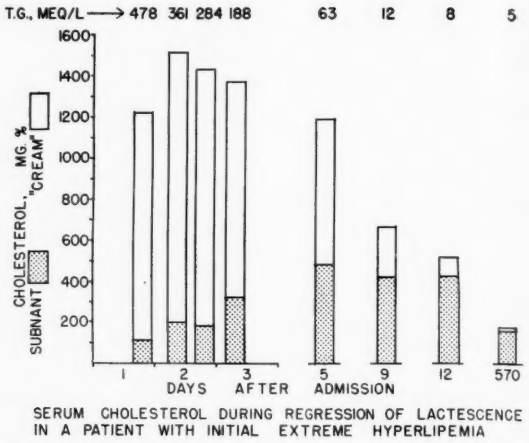

FIG. 2. Total cholesterol distribution between subnatant and cream layers of serum of a diabetic with extreme elevation of triglycerides in lactescent serum. Triglyceride fatty acid concentrations are given at top of figure by numbers horizontally arranged in the line with T.G. mEq./L.

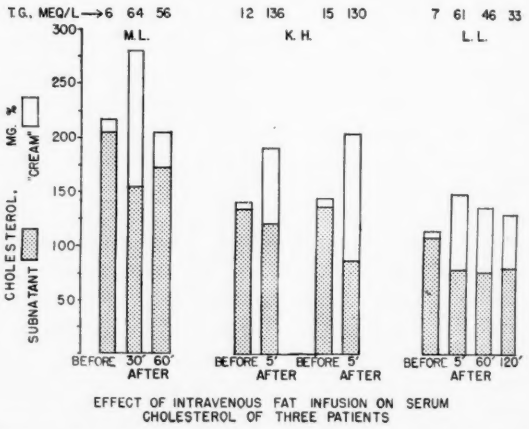

FIG. 3. Total cholesterol distribution between subnatant and cream layers of serum of three patients before and after the intravenous infusion of "Lipomul I.V." (Upjohn). Triglyceride fatty acid concentrations are given at top of figure by numbers horizontally arranged in the line with T.G. mEq./L.

though this has not been a universal finding. Phospholipids were difficult to evaluate because of the rather large amounts of phospholipids in the fat emulsion itself. Havel found increased concentrations of cholesterol and phospholipids as well as triglycerides in the very low density lipoproteins of normal subjects after a fat meal.[23] These observations on man are supported by findings of increased concentrations of serum cholesterol of rats after intravenous injections of emulsified triglycerides or phospholipids.[24] Also these ob-

servations agree with the hypothesis that in nephrosis cholesterol in plasma is trapped by triglycerides.[25]

The cholesterol and phospholipids which are "trapped" or dissolved in the "cream" layer of lactescent sera may be similar to the lipid fraction easily removed by organic solvents without denaturation of the lipoproteins to which the lipids had been attached. This fraction of easily extracted lipids consists of cholesterol in excess of phospholipids, the remaining lipids thus being richer in phospholipids.[29]

In recent years the significance of low density lipoproteins in disease has been investigated.[27] While analyses of others have established the presence of increasing concentrations of triglycerides in lipoproteins of decreasing density,[20] the present analysis places emphasis on the total concentration of triglycerides in serum and thus on the concentration of triglycerides as a factor influencing flotation properties of lipoproteins. This suggests that abnormalities of serum triglyceride content be sought for whenever there are reported to be abnormal, low density lipoproteins. Such a condition has been considered in two diseases, diabetes and atherosclerosis. Although elevations of triglycerides in diabetic acidosis have been recognized for years, the measurement of triglycerides in sera of patients with coronary disease has generally been omitted. Preliminary studies from this laboratory indicate that elevation of triglycerides may be the most common abnormality of serum lipids of patients with recent myocardial infarctions.[6] In a current investigation of almost 100 patients with recent myocardial infarctions, serum cholesterol was above normal in about 25 per cent of the individuals. The serum triglycerides, however, were increased above the normal range in nearly 70 per cent of the persons. Coincidental elevation of triglycerides and cholesterol occurred in only 15 per cent of this group of individuals. Because of this finding, and because of the previously described effect of triglycerides on other lipid fractions, factors influencing the concentration of triglycerides deserve attention.

Reduction of serum albumin[25] or a defect in the clearing mechanism[28] may account for some types of pathological lactescence. Studies of diabetes already outlined suggest that the status of carbohydrate metabolism may be a fundamental factor controlling the concentration of triglycerides in serum not only in diabetes, but in conditions in which abnormally high concentrations of triglycerides occur. The dependence of serum triglycerides on the status of carbohydrate metabolism in diabetes can be demonstrated during even brief periods. In the pre-insulin era Blix[29] described higher fatty acids after a fast day than after a carbohydrate meal. Years ago before the present interest in nonesterified fatty acids diabetic patients maintained on regular insulin exhibited an overnight rise in concentration of serum total fatty acids which accompanied the rise in blood sugar as the effect of insulin abated (figure 4).[30] On the other hand, alimentary lipemia of controlled diabetics was little different from that of normal individuals when carbohydrate metabolism was adequately controlled by insulin.

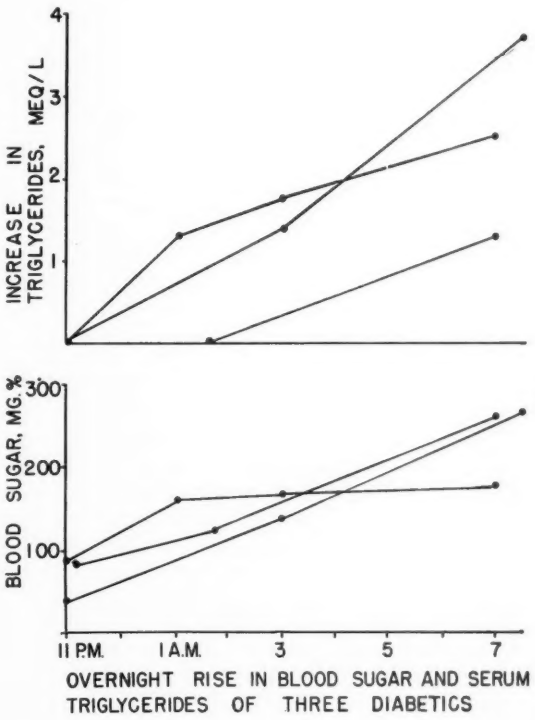

FIG. 4. Overnight rise in blood sugar and serum triglyceride fatty acids of three diabetics receiving regular insulin.

Carbohydrate metabolism has an influence on serum triglycerides in normal individuals as well as in diabetics. Gordon[18] and Dole[17] described the increase in nonesterified fatty acids during starvation and their reduction following glucose administration. Havel et al.[31] have found lower concentrations of triglycerides after a fat-free carbohydrate meal than in the postabsorptive state. In recent experiments from this laboratory a test fat meal containing 60 gm. of fat but no carbohydrate, was fed to a group of normal subjects under two different sets of conditions.[32] On one occasion the group had fasted overnight and was presumably mildly depleted

of carbohydrate. On the other occasion the group was similarly prepared except that carbohydrate metabolism was strongly stimulated by the administration of divided doses of glucose given orally in amounts varying from 100 to 250 gm. over several hours before and after the fat meal. In figure 5 the data show that the usual rise in triglyceride concentration following the test meal was diminished or abolished by the concomitant administration of glucose. A similar reduction of alimentary lipemia can be produced by glucagon injection.[33] Stimulation of carbohydrate metabolism thus not only diminishes mobilization of depot fat, as described by Gordon[16] and by Dole[17] but causes a reduction of circulating triglycerides of dietary origin. A more detailed discussion of the effects on serum lipids of glucose metabolism has been published.[34]

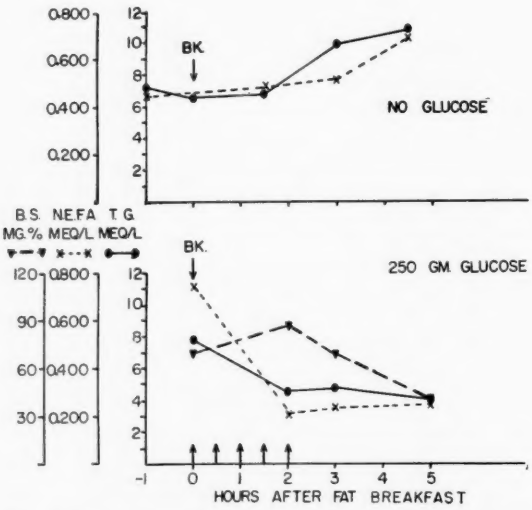

FIG. 5. Blood sugar, triglyceride and nonesterified fatty acids of one normal male subject given two high fat breakfasts, one without glucose and one during the ingestion of 250 gm. of glucose. Reproduced by permission of Metabolism, from Albrink, M. J., Fitzgerald, J. R., and Man, E. B., "Reduction of alimentary lipemia by glucose." Metabolism, in press.

The obvious conclusion from this material that a high carbohydrate diet might obviate the possible adverse effects of prolonged alimentary lipemia or of increased concentrations of plasma triglycerides should not be drawn without due consideration of other factors. To maintain the balance between isocaloric intake and expenditure, when carbohydrate intake is high dietary fat must be reduced. A high carbohydrate, low fat diet necessitates the conversion of large amounts of carbohydrate to fat, a synthesis having the possible disadvantage of requiring insulin, and resulting in the synthesis of fatty acids which are usually saturated.[35] Without a dietary source of unsaturated fatty acids, a deficiency of the latter might occur. A further consequence of a high carbohydrate diet results from the facts that enzyme systems respond effectively to adaptation, and man, in common with most terrestrial animals, does not eat continually, but intermittently. Carbohydrate is rapidly absorbed, burned, and removed from the metabolic pool while fat eaten at the same time is both slowly absorbed and transported into the blood stream.[36] Indeed, adaptation to a diet high in carbohydrate merely speeds up the utilization of administered carbohydrate and exaggerates the rapidity with which stores of glycogen are subsequently depleted.[37] Adaptation to low carbohydrate, high fat diets may have distinct advantages to the organism. An animal so adapted does not squander glucose flagrantly, but conserves it for vital reactions probably concerned with the smooth operation of the Krebs cycle in the absence of exogenous glucose. Such an animal burns fat with more efficiency and consequently survives starvation for a longer period with less ketosis than does a carbohydrate adapted animal.[37,38]

The low carbohydrate, high fat diets used to treat diabetes in the pre-insulin era serve as a human counterpart to these experiments. According to Blix[3] after a transient increase in serum lipids with initiation of such diets the lipids became normal. Even the serum lipids of initially lactescent sera became clear in some patients receiving over 200 gm. of fat and only 30 gm. of carbohydrate a day. In those days a diet containing 150 gm. of fat was considered "low fat" for a diabetic .

When insulin became generally available interest in such high fat diets waned. Relative restrictions of fat and replacement of the calories with carbohydrate became common dietary treatment. Recently, investigations of biochemical adaptation raise the possibility that a high carbohydrate diet may leave the individual ill equipped to metabolize fat which must supply energy during the intervals between feeding. Decisions to treat disorders of lipid metabolism with low fat, high carbohydrate diets might well be reconsidered until more is learned about metabolic and adaptive changes occurring in human beings in response to such diets.[9,23,36]

Further studies must delineate the effect on concentration of serum triglycerides of previous dietary patterns. Studies of metabolic pathways in diabetes reveal that the operative framework of metabolism cannot be compartmentalized into combustion and synthesis of carbohydrate, fat or protein, but must be considered as a whole.

SUMMARY

Lipids in sera of diabetic patients are as a rule normal when diabetes is controlled. With lack of control elevations in concentration of triglycerides are more frequent and of greater magnitude than are increases of cholesterol or phospholipids. With treatment of the diabetes serum lipids rapidly return toward normal.

The effect of serum triglycerides on the concentration and physical state of other lipid components is suggested by studies of pathologically lactescent sera and of sera obtained from patients shortly after intravenous infusion of fat emulsions.

An interrelation between carbohydrate metabolism and serum triglyceride concentration is demonstrable in normal individuals as well as in diabetics.

SUMMARIO IN INTERLINGUA

Triglyceridos Del Sero In Sanitate E In Diabete

Le lipidos in le seros de patientes diabetic es generalmente normal quando le diabete es sub controlo. In le absentia de iste controlo, elevate concentrationes del triglyceridos es plus frequente e plus marcate que elevate concentrationes de cholesterol o del phospholipidos. Le tractamento del diabete resulta in un rapide retorno del lipidos in le sero a nivellos normal.

Un effecto del triglyceridos del sero super le concentration e le stato physic de altere componentes lipidic es suggerite per studios de seros de character pathologicamente lactescente e de seros obtenite ab patientes tractate un breve tempore previemente con infusiones intravenose de emulsiones grasse.

Un interrelation del metabolismo de hydratos de carbon e del concentration de triglyceridos in le sero es demonstrabile in individuos normal e etiam in diabeticos.

ACKNOWLEDGMENTS

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DISCUSSION

ELAINE P. RALLI, M.D.: It is interesting that Dr. Rubin (*J. Biol. Chem. 131*:691-702, 1939) reported studies on an adolescent diabetic girl admitted to Bellevue Hospital in ketosis, in which an elevation in the serum triglycerides was similar to that reported by Dr. Albrink and Dr. Man. Their data and ours show the decrease in all the lipid fractions associated with insulin therapy (table 1). It also emphasizes the fact that the elevation in the lipids was mainly in the triglyceride fraction. Obviously, it requires a considerable period of time for the elevated lipid values to return to normal. In this case, they eventually did. At the time of the elevated serum lipids, the serum was milky, as was pointed out by Doctors Albrink and Man in their cases.

The question of alterations in the lipid fractions is interesting because of its possible relationship to the development of atherosclerosis. In the diabetic patient, when there is distinct elevation in lipids there is usually a marked increase in the serum level of carotene. This is shown in table 1 and we have reported it in a large series of cases. It is significant, too, I think, that the atheromatous plaques in the arteries of diabetic patients are often orange-colored, indicating that they contain carotene. Interestingly enough, in patients with cirrhosis of the liver associated with fatty infiltration of the liver, at which time the serum lipids are usually elevated (Stueck, Rubin, Clarke, Graef and Ralli, *Am. J. Med.* 5:188-201, 1948), the increase in lipids is usually due to an increase in both the phospholipids and the triglyceride fractions.

The use of a limited fat diet in patients with diabetes has been in vogue since 1936. From what we have seen in the clinics, this diet has been associated with a lessening of the tendency of serum lipids to increase.

TABLE 1

Plasma of a 14-year-old diabetic
(All values are expressed in mg. per 100 ml. of plasma, unless otherwise noted)

Days observed	Total lipids	Cholesterol			Total fatty acids		Triglycerides	Phospholipids	Choline	Cephalin*	Water	Carotene
		Free	Total	Per cent free		Iodine number			mM. per cent	per cent	gm. per 100 ml.	
0	22,970	950	1,615	59	19,650	75.3	18,800	2,000				
2	15,160	820	1,380	59	12,620	75.3	11,970	1,280	1.46	22	78.1	0.69
5	8,550	543	1,290	42	6,430	75.6	5,620	1,150	1.30	18	84.4	0.58
7	5,020	335	1,080	31				770				
13	2,580	208	754	28	1,480	90.1	760	550	0.58	23	90.5	0.322
20	1,775	141	543	26	969	82.7	520	285	0.378	3	91.3	
27	1,420	103	346	30	818	79.7	520	226	0.291	6	91.6	0.118
39	1,140	75	260	29	649	92.3	340	299	0.370	9		0.129
88	1,494	104	355	29	892	95.7	520	330	0.321	29		
102	1,547	106	353	30	969		580	364	0.330	34		0.212
137	1,154	92	291	32	631	99.8	287	325				

*Cephalin calculated as per cent of total phospholipids.

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In the early insulin era the fat content of the diet of the diabetic was still as high as 120-140 gm. Now we try to keep most of our patients on a fat intake of 85-100 gm.

One should not, however, infer that the serum lipid level is the sole explanation for the atherosclerosis that is a prominent part of the disease. Other factors undoubtedly contribute to the production of atherosclerosis. I think it is of some interest that the patient with hypothyroidism also tends to have an elevated cholesterol level and an elevation in the serum carotene level. It may be that hypofunction of the thyroid is associated with an increase in serum lipids and that the diabetic patients in whom the serum lipids are high and in whom the carotene is also elevated are suffering from some deficiency of the thyroid hormone.

Hormonal and Nutritional Factors Influencing Cardiac Glycogen

Glycogen in the liver is formed from dietary glucose and other carbohydrate sources and serves as a reservoir of glucose, which is slowly released during the early phase of fasting to maintain the blood sugar. The behavior of cardiac glycogen is paradoxical in that the level rises in normal rats during fasting. Moreover, the significance of cardiac glycogen is obscure in terms of the metabolism of heart muscle. It is known that the heart can function normally during fasting by burning non-carbohydrate nutrients.

J. A. Russell and W. L. Bloom (*Endocrinology* 58:83, 1956), have observed that the normal rise in cardiac glycogen with fasting does not occur in hypophysectomized rats. This suggests the importance of the pituitary in determining the level of cardiac glycogen during fasting. Previously, B. A. Illingworth and J. A. Russell (*Endocrinology* 48:423, 1951), had shown that growth hormone increased the amount of glycogen in the gastrocnemius, heart and diaphragm of fasting normal rats and restored to normal the glycogen levels in these tissues of hypophysectomized rats.

Further observations on the effects of nutrition and growth hormone on cardiac glycogen have been made by G. A. Adrouny and J. A. Russell (*Endocrinology* 59: 241, 1956). Their observations showed the effective dose of growth hormone in hypophysectomized rats to lie between 62.5 and 500 μg., and the response to be essentially a linear function of the dose.

Since feeding a normal diet to a previously fasted rat causes the cardiac glycogen level to fall within twelve hours to the level resulting from normal feeding, the authors decided to investigate the components of the diet to determine which were most significant in lowering cardiac glycogen. Therefore, fasting normal rats were fed diets consisting of isocaloric quantities of either carbohydrate, protein or fat. The greatest drop in cardiac glycogen occurred following the carbohydrate feeding. Protein feeding produced a significant, but smaller, fall and fat feedings were essentially without effect upon cardiac glycogen. The investigators reasoned that the effectiveness of carbohydrate feeding might have resulted either from a decrease in secretion of pituitary factor, or from inhibition of activity of circulating hormone.

The action of growth hormone on cardiac glycogen was shown to be unimpaired by carbohydrate feeding. This was done first by fasting rats, and then by giving them graded doses of growth hormone after the restoration of food. The increment in total glycogen twelve hours after the administration of growth hormone was not significantly different from that which was found in the fasting rats. This would indicate that growth hormone could counteract the glycogen-lowering effect of food. Some doubt on this point must remain because the authors comment that when the period of growth hormone action was prolonged to twenty-four hours, little increase in cardiac glycogen was observed.

The observations provide support for the hypothesis that the rise in cardiac glycogen which occurs with fasting, at least in the rat, is related to the release of a pituitary factor. Although growth hormone preparations possess the ability to raise the level of cardiac glycogen, there is some uncertainty concerning the identity of this activity. No good, direct evidence exists which shows that the level of growth hormone fluctuates inversely with food intake. It is difficult to see how such a pattern of release could be helpful in achieving the primary action of growth hormone, namely increased growth.

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Effect of Dietary Protein upon Fat Transport

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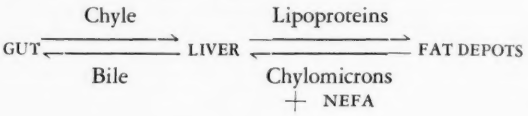
The influence of diet upon the transport of lipids within the mammalian organism has become an increasingly popular and important subject for study during the last few years because of the growing realization that the average concentration of plasma lipids resulting from a given mode of fat transport over a prolonged period of time bears a relationship to the rate of atherogenesis. Of the many constituents of the diet concerned with fat metabolism and fat transport, dietary fat has been singled out as the chief factor relating diet to sustained hyperlipemia and hypercholesterolemia and, as a presumed consequence, to atherosclerosis. The epidemiological studies of Keys and associates[1] relating dietary fat content to mortality from coronary heart disease in various populations have been criticized recently as showing no association[2] between these variables or showing a less impressive association between coronary artery disease and dietary fat intake than with other dietary constituents, particularly animal protein.[2-4] It is my purpose to review the experimental and clinical evidence that protein, particularly animal protein, plays a role in fat transport and may, in certain ranges of intake, play a critical role in determining postabsorptive serum lipid concentrations.

LIPID TRANSPORT

Before we undertake a discussion of the effect of protein upon fat transport, I should like to review very briefly our current concepts of fat transport as they relate to the function of three organs primarily concerned with this process, i.e., the gut, the liver and the fat depots. The level of serum lipids in the mammal under various conditions of diet and metabolic state reflect principally the interaction of these organs in accomplishing the addition of lipid to or removal of lipid from the plasma compartment. In addition, it is probable that dietary and metabolic factors, including, of course, those operative in diabetes mellitus, exert their influence upon fat transport by modifying the function of one or more of these organs. A simplified schema of lipid transport between gut, liver and depots is shown below:



What is immediately apparent from this diagram is the central role which the liver plays in fat transport. Fat and cholesterol are absorbed from the gut and transported essentially quantitatively as chylomicrons via the thoracic lymph[5,6] to the systemic venous blood. These chylomicrons are then rapidly cleared[7] from the blood by elements of the reticulo-endothelial system, particularly the Kupffer cells of the liver[8] and possibly other organs. The fatty acids from the cleared triglycerides appear rapidly in the nonesterified fatty acid fraction (NEFA) which is taken up by many tissues including liver. The cleared cholesterol is apparently transferred to the liver parenchymal cell directly from the Kupffer cell where it becomes available for excretion in the bile (as cholesterol or bile acid) or secretion into the plasma as lipoprotein. In man, this transfer mechanism for dietary cholesterol results in a lag of three days in the attainment of maximum specific radioactivity of the plasma cholesterol after feeding cholesterol-4-C^{14}.[9]

In the steady state the rate of loss of lipid from the liver via oxidation within the organ, net lipoprotein secretion,* and biliary excretion is equal to the gain in lipid via endogenous synthesis plus uptake from the periphery (whether chylomicron lipid directly, via R-E cells, or via the NEFA fraction). In the postabsorptive state, furthermore, these various rates are constant in a given organism and the liver lipid and serum lipid levels are stable. Disturbances in any of these rates may lead to changes in liver and serum lipid content. For example, it is well known that biliary obstruction will elevate the serum cholesterol and other serum lipids. Enhanced synthesis of lipid by the liver occurs in xanthomatous biliary

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* There is an appreciable exchange of serum and hepatic lipoprotein lipid across the liver cell and sinusoidal membranes since both cholesterol and serum phospholipid are synthesized and ultimately catabolized by the liver.

cirrhosis[10] and presumably in other hereditary hypercholesterolemic xanthomatoses. The effect of dietary corn oil in lowering serum cholesterol levels in man appears to depend upon an increased rate of biliary excretion of sterol.[11]

Fatty liver occurs when the rates of lipid entry plus synthesis exceed those of exodus plus oxidation. Homeostatic mechanisms exist to attenuate synthesis of cholesterol when there is excessive entry[12] but these mechanisms apparently do not exist for triglyceride.[13] On the basis of deuterium labeling of newly synthesized fat, Stetten and Salcedo[14] distinguished between (a) fatty livers due to defective transport of lipid from liver to depots and (b) fatty livers due to excessive transport of lipid from depots to liver. Fatty livers resulting from liver disease, including intoxications, protein malnutrition and choline deficiency appear to belong to the first category. These situations are generally associated with hypolipemia and are to be discussed in detail later. Fatty liver resulting from starvation, diabetes mellitus, and anterior pituitary excess are in the second group and are generally associated with hyperlipemia. In uncontrolled diabetes mellitus the mobilization of lipid from the depots is brisk. Not only do nonesterified fatty acids pour out as Dole has observed[15] but also triglycerides appear in the form of chylomicrons.[16] In some instances the hyperlipemia resulting from mobilization of depot fat in uncontrolled diabetes is massive and presents a profile of serum lipids not unlike that seen in idiopathic hyperlipemia. Whereas in diabetes the hyperlipemia results from excessive mobilization of depot fat, in idiopathic hyperlipemia the problem appears to be defective clearing.[17] In some individuals the two disorders appear to be associated.[18] In table 1 are presented some representative data on serum lipids and lipoproteins for diabetic lipemia, idiopathic hyperlipemia and idiopathic hyperlipemia in association with diabetes. In all instances the triglycerides are elevated out of proportion to cholesterol and phospholipid and the plasma is lactescent. Xanthomatosis may be seen in all three conditions and atherosclerosis is particularly prevalent in the groups with idiopathic hyperlipemia. Diabetic lipemia responds well to insulin therapy whereas the lipemia of idiopathic hyperlipemia does not. The latter lipemia responds moderately well to low fat diets, heparin[19] and even to estrogen[20] although no agent suffices to restore lipid transport to normal.

Although the lipid partition in the two types of lipemia are indistinguishable, the lipoprotein partition reveals some interesting differences. In diabetic lipemia the S_{f0-12} fraction is normal and the major elevations are in the S_{f12-20} and $S_{f20-100}$ fractions; in idiopathic hyperlipemia the S_{f0-12} fraction is significantly reduced and the major elevations are in the $S_{f20-100}$ and

TABLE 1

Lipid and lipoprotein patterns in healthy human subjects and in patients with diabetic lipemia and idiopathic hyperlipemia

	Normal*	Diabetic lipemia		Idiopathic hyperlipemia with diabetes		Idiopathic hyperlipemia	
		Case 9† F 15 yrs.	34 Cases‡ Mean	Case JF§ F 53 yrs.	Case 1‖ M 51 yrs.	Case 20¶ F 60 yrs.	9 Cases** Mean
Lipids							
Total lipid	690	—	2,490	1,140	4,470	3,880	—
Triglyceride	150	—	1,310	464	3,231	2,462	—
Cholesterol	220	560	570	313	594	630	—
Phospholipid	250	—	410	276	645	587	—
Lipoproteins							
S_f 0-12	370	390	—	175	—	163	229
S_f 12-20	50	170	—	244	—	78	66
S_f 20-100	100	1,310	—	376	—	867	450
S_f 100-400	50	390	—	60	—	1,300	967

*Typical values for healthy Americans of middle age.[45]

†A fifteen-year-old diabetic girl in acidosis, blood sugar 268, CO_2 9 mEq./L.[46]

‡The mean of thirty-four cases of severe diabetes with negligible carbohydrate tolerance and with mild to moderate acidosis in the pre-insulin era.[47]

§A fifty-three-year-old white female with nonketogenic diabetes requiring 40 units of insulin daily and obesity, tuberous xanthomatosis and cerebral artery occlusion. Serum lipids declined after treatment with 1,200-calorie diet containing 50 gm. of fat. (Case D-6380 Presbyterian Hospital, University of Pittsburgh Medical Center, Pittsburgh.)

‖A fifty-one-year-old white male with nonketogenic diabetes, obesity, lipemia retinalis, eruptive xanthomatosis, angina and old myocardial infarction. Serum lipids declined after treatment with 1,200-calorie low-fat diet.[38]

¶A sixty-year-old female with papular xanthomatosis.[39]

**The mean of nine patients (two females, seven males) with idiopathic hyperlipemia but no cutaneous lesions.[48]

$S_{f100-400}$ fractions. When the two disorders are associated, the clearing defect appears predominant. This is apparent from the lipoprotein spectra manifested in Case JF as well as the refractoriness of these patients to treatment with insulin. In diabetic lipemia, where clearing is normal the liver is usually fatty due to the tremendous influx of peripheral lipid. In idiopathic hyperlipemia, liver fat does not accumulate. This fact, coupled with the reduced "normal" S_{f6} beta-lipoprotein secretion by the liver in this disorder suggests that the liver may be critically involved in the clearing defect.

Since the liver is the source of serum cholesterol and phospholipid, it seems certain that the hypercholesterolemia and hyperphospholipidemia which accompanies the lipemia in both conditions is part of the hepatic response to increased triglyceridemia. In fact, Friedman and Byers[21] have shown that the infusion of neutral fat emulsions into rats causes a marked increase in their serum cholesterol level. Seifter and Baeder[22] described a lipid-mobilizing factor (LM) from the plasma of animals given cortisone which caused hyperlipemia and hypercholesterolemia in control fasted rats but only hypertriglyceridemia in fasted hepatectomized rats. Hepatectomy abolished the usual response to hyperchylomicronemia. It seems probable, therefore, that in physiological states characterized by persistently high levels of circulating chylomicrons (whether in diabetes, idiopathic hyperlipemia, or high fat feeding) the usual response is an increased elaboration of β-lipoproteins by the liver which secondarily elevates the serum cholesterol and phospholipid levels and may thus contribute to the progression of atherosclerosis.

DIETARY PROTEIN AND FAT METABOLISM

From the foregoing, it is apparent that dietary factors which influence the metabolism of fat by the liver, and especially those which influence the secretion of β-lipoproteins by this organ, could be of prime importance in the pathogenesis of atherosclerosis. Dietary protein, or more specifically the amino acids which are supplied by dietary protein, are involved in the maintenance of all tissues but particularly the gut and the liver, and are known to participate in a number of biochemical reactions of importance in fat metabolism and fat transport. Some of general effects of adequate dietary protein include (a) the maintenance of nitrogen balance, (b) the maintenance of the integrity of the gastrointestinal epithelium and hence its capacity for the absorption of fat, and (c) the maintenance of normal liver structure and function. More specific roles for given amino acids include a role of cystine as a source of the thioethanolamine moiety in coenzyme A formation (required in all acetyl transfer reactions involved in lipid anabolism and catabolism), and as a source of taurine for taurocholate formation. Methionine is required for the synthesis of choline and choline is required for metabolism of fat from the liver and the elaboration of serum lipoproteins as shall be discussed later. Even in the presence of adequate choline, imbalance of intakes of methionine, lysine, tryptophane and threonine in a given protein may cause fatty liver in experimental animals.[23] Finally, amino acids are required for the synthesis of the peptide moieties of the serum lipoproteins but the extent to which they are limiting for this purpose in given experimental or clinical situations is not known.

In severe protein malnutrition in man there is weakness, weight loss, wasting, edema, hypoalbuminemia, hepatomegaly, gastrointestinal mucosal atrophy and negative nitrogen balance. The adipose depots are generally reduced, the serum lipids are low normal or reduced, and the liver fatty. The most dramatic manifestations of protein malnutrition are seen in weanling children in which the disease is called kwashiorkor.[24] In this disease there appears to be both defective fat absorption, due to alteration in the intestinal mucosa, and defective mobilization of lipid from the liver. The serum lipids are low (cholesterol circa 80 mg. per cent) and are restored toward normal in a few days by feeding fat-free milk powder. This phenomenon precedes by some time the return of the gastrointestinal tract to a normal capacity to absorb fat. The feeding of inadequate proteins to animals also results in relative or absolute hypolipemia. This is particularly true if choline deficiency is also imposed. This was observed by McKibbon et al.[25] in puppies, Mann et al.[26] in Cebus monkeys, and Ridout et al.[27] in rats. In man, monkey, dog and rat, therefore, protein deficiency possibly as it influences the availability of lipotropic factors, appears to result in hypocholesterolemia.

EFFECT OF ALIPOTROPIC NUTRITION ON SERUM LIPIDS AND LIPOPROTEINS OF THE RAT

Three years ago we undertook a study of the effect of low methionine, low choline diets upon the serum lipids and lipoproteins of the rat and more recently of man. The remainder of this paper deals largely with these observations, which are reported in detail elsewhere.[4]

Young Sprague-Dawley male rats of the Holtzman strain weighing about 120 gm. were fed experimental diets representing the permutations of low/high methionine, low/high choline, low/high fat, low/high unsaturated fat and high/low cholesterol. The range of the dietary content of methionine was 0.16 per cent (soy protein) to 0.54 per cent (casein). The range of the con-

tent of choline was 0-0.3 per cent; the range for fat was 6-40 per cent by weight; the range for dietary cholesterol 0-1 per cent. The level of protein was 18 per cent with soy protein providing the low-methionine level and casein the high-methionine level. The total organic sulfur of these rations was kept constant at 0.2 per cent by varying the content of cystine. Lard, butterfat, and corn oil were used as sources of fat.

Each group of twelve rats was fed the experimental ration for two weeks and sacrificed by needle aortotomy under nembutal anesthesia. Serum lipids and lipoproteins were determined on pooled samples of serum from subgroups of four animals. Serum total cholesterol, phospholipid and total lipid (after saponification) were measured chemically[28] and triglyceride and total lipid (before saponification) calculated from these data. The high density lipoproteins (corresponding to the α-lipoproteins of the electrophoretogram) and low density lipoproteins (corresponding to the β-lipoproteins of the electrophoretogram) were measured by the ultracentrifugal method of Gofman.[29] Liver fat, and in some experiments liver cholesterol, were also determined.

The results of these experiments are presented in tables 2, 3, and 4. The findings in animals fed given experimental diets are also compared with stock animals fed Purina chow in each table. The rat characteristically has lower levels of serum lipids than man[30] and an appreciably different profile of serum lipoproteins.[31] Rats have approximately the same amount of high-density α-lipoproteins as man, but the concentration of all of the low-density β-lipoproteins is significantly reduced with none appearing normally in the S_{f12-20} range. Animals fed the soy protein rations devoid of choline and low in methionine developed markedly fatty livers and generalized hypolipemia when compared with either the animals fed the stock ration or controls fed the same experimental ration plus choline. Serum cholesterol, phospholipid and triglyceride all declined and the low-density β-lipoproteins virtually disappeared. Elevating the fat content of the soy protein diet from 6 to 40 per cent in the absence of choline had no appreciable effect upon the distribution of serum lipids or β-lipoproteins. The addition of choline to the soy protein rations prevented the hypolipemia, the hypobetalipoproteinemia, and resulted in slightly higher values for phospholipid, triglyceride and high-density α-lipoproteins than found on the stock ration.

The use of casein as a source of protein without added choline (diet 5) minimized the hypolipemia and hypobetalipoproteinemia seen in rats fed the choline-deficient soy protein diets (viz. diet 3) although it did not completely abolish it. It is assumed that the extra methionine contributed its methyl group for choline synthesis, and this is borne out by the lesser fatty infiltration of the liver observed in these animals. The less dramatic effect of low choline rations upon the serum lipoproteins of rats studied by Wilgram, Lewis and Blumenstein[32] is probably due to the more generous amounts of methionine supplied by their ration. In further support of the role of methionine in lipoprotein secretion by the liver is the observation by Feinberg et al.[33] that marked hypolipemia and hypolipoproteinemia is seen in dogs given ethionine, an antagonist of methionine.

The use of butterfat or corn oil in place of lard (see table 3) did not influence the results. Neither in the presence or absence of choline was the hypolipemic effect of unsaturated dietary fat observed in man[34-36] noted in these rats.

The results of feeding animals supplementary cholesterol with taurocholate, to enhance absorption, with and without added choline are presented in table 4. As previously noted, feeding the alipotropic diet resulted in a generalized hypolipemia, marked fatty infiltration and some increase in liver cholesterol. The addition of choline prevented all of these changes. When 1 per cent cholesterol plus 1 per cent sodium taurocholate was added to the choline-deficient diet the hypolipemia persisted despite a large increase in liver cholesterol. The addition of choline to the cholesterol-supplemented ration decreased the accumulation of cholesterol in the liver and resulted in hypercholesterolemia and hyperlipemia. It would appear that the presence of choline in the diet is essential for the manifestation of hypercholesterolemia even when the source of the cholesterol is dietary. The reason for this paradox appears to be that absorbed cholesterol appears briefly but quantitatively in the chylomicrons which are rapidly cleared by the hepatic Kupffer cells and possibly other elements of the reticuloendothelial system. The sterol is then transferred to the live parenchymal cell where it becomes available for secretion into the plasma as a lipoprotein or degradation and excretion as a bile acid as previously noted. In the choline-deficient rat it would appear that the secretion of lipoprotein cholesterol by the liver is reduced whether the sterol is of exogenous or endogenous origin. This has also been noted by Wilgram et al.[37]

RELEVANCE TO MAN

The relevance of these findings in the rat to man is an important question. One cannot transpose data from one species to another without direct evidence. In another primate, the Cebus monkey, however, Mann et al.[28] found that hyperlipemia from cholesterol feeding was not

TABLE 2

Effect of dietary choline, methionine and fat upon the serum lipids and lipoproteins of rats

	Diet			Liver lipid	Serum lipids mg. per cent				Serum lipoproteins mg. per cent					
									High Density	Low Density (β)				
No.	Lard per cent	Meth. per cent	Choline per cent	Total per cent	Cholesterol	Phospholipid	Triglyceride	Total	(α)-S_6	S_f 0-12	S_f 12-20	S_f 20-100	S_f 100-400	Total
Chow				4.1	90	139	30	311	125	38	0	37	14	89
1	6	0.16	0.0	30.1	49	95	7	174	90	2	0	2	0	4
2	6	0.16	0.3	5.9	87	188	94	410	150	28	0	62	74	164
3	40	0.16	0.0	35.6	50	126	8	207	147	0	0	0	0	0
4	40	0.16	0.3	6.2	93	184	86	406	195	37	0	38	13	88
5	40	0.54	0.0	22.9	88	165	12	306	120	22	0	18	15	65
6	40	0.54	0.3	5.8	90	166	54	352	158	38	0	28	8	74

TABLE 3

Effect of type of fat on the serum lipids and lipoproteins of rats fed low-methionine diets with and without choline

	Diet		Liver lipid	Serum lipids mg. per cent				Serum lipoproteins mg. per cent					
								High Density	Low Density (β)				
No.	Fat*	Choline per cent	Total per cent	Cholesterol	Phospholipid	Triglyceride	Total	(α)-S_6	S_f 0-12	S_f 12-20	S_f 20-100	S_f 100-400	Total
Chow			4.1	90	139	30	311	125	38	0	37	14	89
3	L	0.0	35.6	50	126	8	207	147	0	0	0	0	0
4	L	0.3	6.2	93	184	86	406	195	37	0	38	13	88
7	Bu	0.0	29.1	56	102	20	204	119	3	0	1	0	4
8	Bu	0.3	8.5	99	171	91	409	181	23	0	31	9	63
9	CO	0.0	26.5	59	104	28	220	142	2	0	0	0	2
10	CO	0.3	8.9	106	177	94	427	210	28	0	35	14	77

*Level of dietary fat in all experiments 3-10 was 40 per cent by weight; L = lard; Bu = butterfat; CO = corn oil.

TABLE 4

Effect of cholesterol and taurocholate feeding upon the serum and liver lipids of rats fed low-methionine diets with and without choline

	Diet*		Liver lipids gm. per cent		Serum lipids mg. per cent			
No.	Supplement†	Choline per cent	Cholesterol	Total	Cholesterol	Phospholipid	Triglyceride	Total
Chow			0.25	4.1	90	139	30	311
11	0	0.0	0.56	23.7	60	108	12	210
12	0	0.3	0.18	3.5	102	168	45	365
13	+	0.0	3.20	30.9	82	105	8	234
14	+	0.3	1.76	8.2	134	165	114	476

*All diets contained 18 per cent soy protein (Drackett C-1) and 20 per cent lard.
†The supplement consisted of 1 per cent cholesterol plus 1 per cent taurocholate.

obtained unless choline was added to the diet. Furthermore, the rice diet introduced by Kempner[38] for the treatment of hypertension in man is one of the most potent hypocholesterolemic rations known. It contains 25 gm. of protein (of which methionine is the limiting amino acid), 5 gm. of fat and carbohydrate in quantity sufficient to maintain caloric balance. Although the hypolipemic effect of this ration has been attributed to its low fat content, the decreases in serum cholesterol,[39,40] are out of proportion to what has been observed on diets as low in fat but replete in protein.[41]

We have studied the effect upon serum cholesterol of feeding low protein vegetable diets to human subjects for short periods.[42] Nine subjects, five of whom were hypercholesterolemic, were fed a control diet containing 100 gm. of protein, 80 gm. of fat (*ca.* 36 per cent of calories) and 300-350 gm. of carbohydrate for a period of one to two weeks. The protein was derived mainly from animal sources and supplied the essential amino acids in amounts from four to eight times the estimated human requirement.[43] The dietary fat was likewise derived chiefly from animal sources. At the end of the

control period the subjects were fed an isocaloric isofat diet (including 50 gm. butterfat) containing 25 gm. of vegetable protein derived from cereal, rice and legumes for one week. All essential amino acids except methionine were supplied by the low protein diet in amounts meeting Roses' tentative minimum requirement and all other essential nutrients were given in adequate amounts. The low protein diet contained about 0.20 gm. of choline as compared to about 1.0 gm. in the control ration. Serum cholesterol was measured twice weekly and serum lipoproteins once weekly in some cases. At the end of the low-protein period, the subjects were again fed the control diet. All subjects showed a decrease in serum cholesterol during the low-protein period which averaged 44±4 mg. per cent as shown in table 5 and all values returned toward normal upon resumption of the control diet. The changes in body weight were nil during these relatively short term studies.

One of these subjects, AK, was studied in considerably more detail and for a longer period (ten weeks) on the low-protein regime. In table 6 are presented the changes in serum lipids and lipoproteins resulting in this man after four weeks of feeding the low protein diet at which time the lipids were stabilized. The changes are not as dramatic as those seen in the rat but they are identical in kind. All of the serum lipids and all of categories of β-lipoproteins are decreased, despite the continued intake of sizeable amounts of saturated fat. There were no other changes in liver function as measured by the usual battery of tests. These human studies would support the view that methionine and/or choline is a critical metabolite for the maintenance of normal serum lipoprotein levels in man as well as in the rat, dog and monkey. The study of dietary protein in relationship to serum total cholesterol and β-lipoprotein cholesterol by Keys and Anderson[4] was inconclusive, probably because of the relatively high range of dietary protein (65-138 gm.) studied. With regard to epidemiological studies of heart disease in relation to diet, Yerushalmy and Hilleboe[2] and Yudkin[3] have pointed out that the correlation is better with animal protein (which is the best source of methionine) than with total fat. This, of course, does not prove that the intake of dietary fat has no bearing on the pathogenesis of coronary artery disease any more than it proves that animal protein is the critical factor. Epidemiologic associations are never proof of causation; by the same token a role for animal protein has not been excluded by the data.

SUMMARY

In the mammal the liver is the central organ in fat transport by virtue of its uptake of chylomicrons from gut and fat depots, its transformation of NEFA's, its excretion of cholesterol as sterol and bile acids, its capacity for the synthesis of cholesterol and phospholipid, and its secretion of serum lipoproteins. Dietary factors which modify serum lipid concentrations probably do so by modifying the function of the liver. Dietary protein acting chiefly via the balance of the amino acids methionine, cystine, threonine, tryptophane, and lysine (plus choline) is known to influence the function of the liver, its fat content, and its ability to maintain normal concentrations of the serum lipids.

When young adult rats are fed diets low in methionine and choline a marked hypocholesterolemia, hypolipemia and hypolipoproteinemia develop. These effects are prevented by the addition of 0.3 per cent choline chloride

TABLE 5
Effect of low-protein diets upon serum cholesterol in man

Period	Patients No.	Diet Prot.	Fat	CBH	Calories	Body wt. Kg.	Serum cholesterol mg. per cent Mean	Range	Change
Control	9	100	80	320	2,400	61.4	311	215-460	
Experimental	9	25	80	395	2,400	61.2	267	160-420	—44±4

TABLE 6
Effect of a low-protein, low-choline diet upon serum lipids and lipoproteins in man

AK Male 41 years

Diet		Period	Serum lipids mg. per cent				Serum lipoproteins mg. per cent Low Density (β)			
	Protein gm.	Wks.	Cholesterol	Phospholipid	Triglyceride	Total	S_f 0-12	S_f 12-20	S_f 20-100	S_f 100-400
Control	100	2	258	245	99	723	409	22	79	17
Experimental	25	4	213	217	40	570	324	10	35	8

to the diet and are partially prevented by casein presumably through its increased methionine content. The level and type of dietary fat (ranging from 6-40 per cent and including butterfat, corn oil and lard) does not modify this effect of choline (or methionine) upon serum lipids in this species. The hypercholesterolemia which ordinarily results from cholesterol-taurocholate feeding in the rat does not occur in animals fed a low-methionine low-choline diet.

Protein deficiency in man, dog and monkey also produces relative or absolute hypocholesterolemia and hypolipemia. In controlled studies in our laboratory as short a period as one week of feeding a diet containing 25 gm. of vegetable protein resulted in a significant decrease in serum cholesterol and β-lipoprotein levels. A review of the existing clinical, experimental and epidemiologic data on man would suggest that dietary animal protein plays a role in fat transport and may be a significant environmental factor in the development of atherosclerosis.

SUMMARIO IN INTERLINGUA

Effecto De Proteina Dietari Super Le Transporto De Grassia

In mammales le hepate es le organo central in le transporto de grassia. Le activitates del hepate in iste respecto include le acceptation de chylomicrones ab intestino e depositos grasse, le transformation de non-esterificate acidos grasse, le excretion de cholesterol como sterol e acidos del bile, le synthese de cholesterol e phospholipidos, e le secretion de lipoproteinas seral. Factores dietari que modifica le concentration de lipidos in le sero exerce lor effecto probabilemente per modificar le function del hepate. Il es cognoscite que proteina dietari, que age principalmente via le balancia del amino-acidos methionina, cystina, threonina, tryptophano, e lysina (plus cholina), exerce un influentia super le function del hepate, su contento de grassia, e su capacitate de mantener normal concentrationes del lipidos seral.

Quando juvene rattos adulte recipe dietas a basse contento de methionina e cholina, le effecto es un marcate grado de hypocholesterolemia, hypolipemia, e hypolipoproteinemia. Iste effectos es prevenite per le addition de 0,3 pro cento de chloruro cholinic al dieta. Illos es prevenite in parte per caseina, presumitemente in consequentia del augmentate contento de methionina. Le nivello e le typo del grassia dietari—testate in quantitates de inter 6 e 40 pro cento de grassia butyric, oleo de mais, e lardo—non modifica le mentionate effecto de cholina (o de methionina) super le lipidos seral in le ratto. Le hypercholesterolemia, que occurre normalmente in rattos in consequentia de alimentation con taurocholato de cholesterol, non se manifesta in animales que recipe un dieta a basse contento de methionina e de cholina.

Carentia de proteina produce etiam in humanos, canes, e simias un relative o absolute hypocholesterolemia e hypolipemia. In studios controlate executate in nostre laboratorio, un periodo de non plus que un septimana de dieta a un contento de 25 g de proteina vegetal resultava in un reduction significative del nivellos de cholesterol e lipoproteina beta in le sero. Un revista del existente datos clinic, experimental, e epidemiologic relative al homine pare indicar que proteina animal in le dieta ha un rolo in le transporto de grassia e es possibilemente un importante factor ambiental in le disveloppamento de atherosclerosis.

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DISCUSSION

OLAF MICKELSEN, PH.D.: Dr. Olson has re-emphasized the importance of nonlipid dietary factors in controlling the blood cholesterol levels. As he has pointed out (*Am. J. Pub. Health* 47:1537, 1957) and as others have stressed (I. H. Page et al., *Circulation* 16:163,

1957), it is very likely that a variety of factors, dietary as well as nondietary, may be involved in the development of atherosclerosis. In order to secure a definitive answer as to the cause of atherosclerosis in man, it will be necessary to bear in mind the multiplicity of factors that may interact to produce the lesion.

The work of Dr. Olson and his group emphasizes the fact that even though adequate dietary choline produces normal lipid levels in the livers of rats, the serum cholesterol levels may be higher than those seen in animals kept on a choline-free diet. One significant point that should be stressed is the observation of Ridout and co-workers (*Biochem. J.* 58:306, 1954) to the effect that it was only the rats on the choline-free diets that showed reductions in serum cholesterol levels. The addition of even small amounts of choline to the diet produced serum cholesterol levels as high as those seen with larger amounts of dietary choline. Furthermore, the Toronto group also showed that the effect of choline in increasing the serum cholesterol level in rats was apparent if the blood samples were taken two to four hours after the feed was removed from the animals. If a longer period intervened between the last feeding and the blood sample (eighteen hours), the choline-supplemented rats had serum cholesterol levels that were comparable to those of the choline-deficient animals. By a number of tests these investigators were led to conclude that the serum cholesterol level is related to the amount of cholesterol being absorbed from the gut when choline is present in the food.

The role of a low protein diet in reducing the serum cholesterol level is intriguing since, as Dr. Olson has shown, one can secure a good correlation between the per capita intake of animal protein and the mortality from degenerative heart disease (see also J. Yerushalmy and H. E. Hilleboe, *N. Y. State J. Med.* 57:2343, 1957). Suggestive substantiation for such a relation comes from Dr. Olson's earlier report (*J. Clin. Invest.* 36:917, 1957) that the serum cholesterol level could be reduced by lowering the level of dietary protein. Earlier, Kempner (*Am. J. Med.* 4:545, 1948) noticed that patients on a rice diet that provided about 20 gm. of protein per day showed a reduction in serum cholesterol levels. In contrast to these observations is the report of Keys and Anderson (*Am. J. Clin. Nutr.* 5:29, 1957) that dietary protein level had no influence on the serum cholesterol level. It should be pointed out that the Minnesota investigators used 63 gm. as a low level of protein intake. Since 63 gm. of protein should provide a reasonably adequate intake for an adult, levels considerably below that might influence serum cholesterol levels.

Although the relation between various dietary factors and the development of atherosclerosis appears cloudy at the moment, the forecast calls for even murkier weather before the relationships become clarified. The work of Harper, Elvehjem, and co-workers on the influence of various amino acids on the level of fat in the liver (*J. Biol. Chem.* 214:677, 1955; 219:327, 1956) may possibly have some bearing on this problem of atherosclerosis, but to my knowledge nothing has been done on blood lipids. They showed that when rats were fed low-protein diets adequate in methionine and choline, the addition of certain amino acids increased the level of liver fat. Threonine and a number of other substances were active in restoring high liver fat levels to normal.

One cannot leave this subject without a few words of caution. The tremendous emphasis on the factors which influence the serum cholesterol level make it appear as though that measurement were an absolute prognosticator of future cardiovascular events. Although patients with diseases characterized by elevated serum cholesterol levels have a higher than normal incidence of cardiovascular disability, there are still many patients who develop the disability with relatively low serum cholesterol levels (*Circulation* 14:691, 1956). The relatively short-term experiments in which highly abnormal diets are fed either to animals or to man should be used only as guides for exhaustive studies on man under more nearly normal conditions. And, finally, even though a good correlation is found between two variables, this cannot be construed as a cause and effect relation. As J. Yudkin (*Lancet* 2:155, 1957) has pointed out, a much better correlation exists between the yearly increase in coronary mortality in the United Kingdom and the number of radio and television licenses issued than for any of the dietary components. No one, at this stage, would even suggest that such a relationship had a biological significance.

DR. OLSON: I would just like to make one comment for those who fear that this kind of dietary treatment of man (i.e., 25 gm. of vegetable protein daily) inevitably results in fatty liver.

I did not mention the fact that we have followed liver fat histologically by liver biopsy throughout these studies and that man appears to be much more resistant to fatty liver than the rat under given dietary conditions. When the 25 gm. protein diet (5 per cent protein) is fed to rats, they develop a moderately fatty liver in one to two weeks, accompanied by hypocholesterolemia. In our human subject, even though we did find hypolipemia and hypocholesterolemia, there was no increase in the stainable liver fat in ten weeks.

Action of Insulin and Cortisone on Adipose Tissue

Franz X. Hausberger, M.D., Philadelphia

The now classical work by Schoenheimer and Rittenberg has shown the rapid turnover and continuous synthesis of body fat. Drury[1] demonstrated that insulin is essential for fat synthesis. Subsequent work by Stetten et al.[2] and by Chaikoff et al.[3] indicated the liver as the main site of lipogenesis. The newly synthesized hepatic fatty acids were thought to be transported by the blood stream to the fat stores, that is, adipose tissue, in order to be deposited.

No attention was paid in these early experiments with isotopes to the fact that liver contains only one-twentieth of the amount of fat found in adipose tissue. Considering the vastly larger dilution of newly synthesized fatty acids in adipose tissue, one can calculate from in vitro experiments that adipose tissue synthesizes per weight unit at least ten times as much fatty acids from glucose as liver does. Taking into account that fatty tissue contains but one-fifteenth to one-twentieth of the amount of protein found in liver, the lipogenic capacity of the fat cell is truly remarkable.

Table 1 compares some of our findings with results from other laboratories. The results obtained with our in vitro investigations are fully supported by in vivo experiments in which normal as well as eviscerated animals were used. The results obtained from eviscerated preparations may be regarded with some criticism. Favarger and Gerlach,[7] however, employed physiological conditions. The animals were sacrificed twelve minutes after the intravenous glucose injections. Only minimal amounts of hepatic fatty acids could have reached the fat depots under these conditions. Lipogenesis by liver probably plays only a supplementary role to that of adipose tissue. Enhanced lipogenesis is always seen during periods of accelerated fat deposition, at least if a diet is fed which does not contain excessive amounts of fat. Large amounts of dietary fat suppress lipogenesis; fat free diet enhances it greatly.[6] This finding suggests a continuous need of fatty acids as fuel for the body.

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From the Daniel Baugh Institute of Anatomy, Jefferson Medical College of Philadelphia, Philadelphia, Pennsylvania.

In fed rats, insulin accelerates both hepatic and adipose tissue lipogenesis. Figure 1 compares hepatic and adipose tissue lipogenesis in *fasted* rats, made severely hypoglycemic by administration of large doses of insulin. Hepatic lipogenesis ceases completely and can not be reinstituted by raising the glucose concentration in the medium as high as 400 mg. per cent. Adipose tissue lipogenesis is increased above the norm under this condition.[9]

Lipogenesis is practically abolished in tissues of *alloxan diabetic* rats, and can not be induced even if the glucose concentration in the medium is raised above 1,000 mg. per cent. Oxidation, on the other hand, does respond to increase in glucose levels. At glucose concentrations prevailing in vivo, 400 mg. per cent for instance, tissues of rather severely diabetic rats show a rate of oxidation of the same order of magnitude as normal tissues at 100 mg. per cent.[8]

This observation casts some doubt upon the concept that insulin regulates glucose utilization *only* by facilitating its entrance into the cells.

Figure 2 demonstrates the effect of adding insulin to hepatic and adipose tissues. Insulin stimulates rat adipose tissue directly. The rate of adipose tissue lipogenesis may be accelerated by several hundred per cent, as is similarly seen after pretreatment of rats with insulin.[5]

Insulin treated rats, as we will see later, increase adipose tissue weight about four times as fast as controls. Here, I would like to stress, this increase is due to a true growth of adipose tissue. Measurement of the size of the fat cells, which is rather easily done due to their almost spherical shape, shows an abundance of small, apparently young fat cells. There is, in addition, some increase in cell size, possibly more so in the older cells. The protein content of adipose tissue in insulin-induced adiposity remains essentially the same, or decreases only slightly.[10,11]

This true growth of adipose tissue is in contrast to the changes seen in hypothalamic hyperphagia. In hypothalamic obesity, the enhanced fat deposition is nearly entirely due to an enlargement of the fat cells. The cell volume increase almost parallels that of the adipose tissue

TABLE 1

Lipogenesis from radioglucose

	In Vitro			In Vivo
	Rats, stock diet Krebs-Henseleit buffer, 400 mg. per cent glucose, 37 degree Celsius, 3 hours		Favarger et al. 1955[7]	*Mice,* fasted, refed with COH rich diet
	Liver	Adip. tissue		Ratio: activity of adipose tissue fatty acids to activity of hepatic fatty acids
Hausberger et al. 1954[4]	0.02*	1.02*		per weight unit tissue
Hausberger 1957[5]	0.07*	0.85*		average 13.6 (1.0-38.0)
	Rats, high COH diet			*Rats,* high COH diet
	Liver	Adip. tissue	Masoro, Chaikoff et al. 1949[8]	glucose subcutaneously, per cent incorporated into body fatty acids
Hausberger et al. 1955[6]	1.24*	7.8*		Controls eviscerated
Masoro, Chaikoff et al. 1950[8]	1.25*			2.0 1.5 3.0 4.2

*Per cent added glucose carbon recovered in fatty acids per weight unit of liver or adipose tissue.

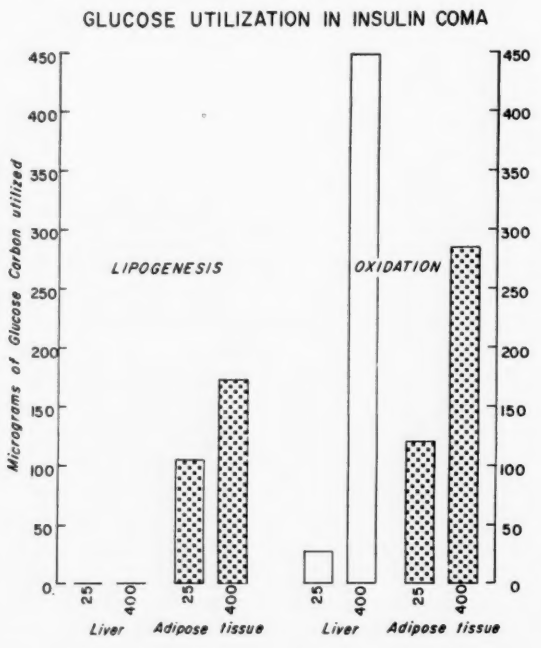

FIG. 1. Glucose utilization per weight unit of liver and adipose tissue of six severely hypoglycemic rats. The fasted animals received repeated injections of Protamine Zinc insulin (each 40 U) within three to four hours. The experiment was performed when convulsions were evident. The average blood glucose level at this time was 25 mg. per cent. The glucose concentrations employed were 25 mg. per cent and 400 mg. per cent.[9]

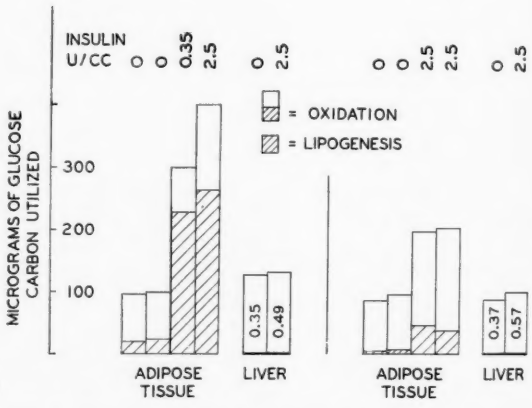

FIG. 2. Glucose utilization by liver and adipose tissue in vitro, without and with addition of insulin. Pooled tissues from three rats, i.e., 200 mg. from each, were used in each experiment. The values for hepatic lipogenesis are expressed by numbers.[5]

mass. The protein content decreases, but to a lesser degree than the cell size increase, indicating that the individual fat cells gain some additional protein.[12]

In the following, I will attempt to demonstrate that insulin is able to overcome some catabolic effects of insulin antagonists on adipose tissue. The results of our investigations indicate that at least certain insulin antagonists, like cortisone, stimulate release of increased amounts of insulin which counteract their action.

Certain adrenal corticosteroids have been shown to

diminish lipogenesis in rats.[13,13a] In other nondiabetic species, however, including man, hyperadrenocorticism does not necessarily reduce glucose utilization. Silvestrini et al.[14] and Bastenie et al.[14,15] concluded from the behavior of the respiratory quotient and "glucose assimilation coefficient," respectively, that cortisone may even increase glucose utilization in (nondiabetic) humans. MacBryde and de la Balze[16] noted increased arteriovenous blood sugar differences after administration of cortical extract to patients with Addison's disease. Welt et al.[17] observed higher rates of glucose oxidation than in controls in two out of three groups of rats receiving cortisone. Hausberger and Hausberger[18] and Sturtevant[19] found no impairment of glucose utilization in steroid diabetic guinea pigs. Finally, Zomzely and Mayer[20] report greatly accelerated lipogenesis from acetate in fed and fasted mice bearing ACTH-secreting anterior pituitary tumors; these animals exhibit hyperglycemia and obesity.

It seems unlikely that any of the known corticosteroids produces obesity directly, or increases glucose utilization. All of the C-11-oxygenated corticosteroids investigated diminish the already impaired glucose tolerance in diabetic humans and animals. Another, indirect, mechanism is suggested by the islet hypertrophy seen in many forms of hyperadrenocorticism, which indicates increased release of insulin.

In order to elucidate the combined effect of hyperadrenocorticism and hyperinsulinism, cortisone or insulin, or both hormones together, were administered to rats and some metabolic parameters were studied. Male Wistar rats, having a body weight of 240 gm., were used. One group of rats was allowed to grow normally for fourteen days without treatment. Other groups received cortisone or Protamine Zinc insulin, or both hormones simultaneously.

The changes in body composition induced by the aforementioned experimental conditions are shown in figure 3. The control rats increased body weight, fat and protein content above that found in animals weighing 240 gm., as shown in the first part. The changes in body fat reflect closely the weight changes of dissectable adipose tissue. Animals receiving 12 units of Protamine Zinc insulin per day accumulated within the same time period about four times as much excess fat. Administration of 5 mg. of cortisone per day produced variable results, which are partly due to infections, rather frequently observed in these animals. In about half of the rats fat deposition was normal although accumulation of body protein was diminished. After administration of both insulin and cortisone together, the effect of insulin was

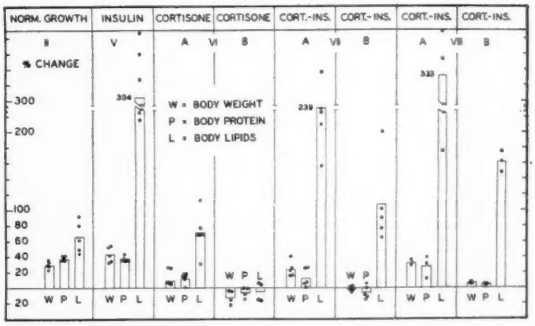

FIG. 3. Changes in body composition of controls and of rats receiving insulin or cortisone, or both hormones simultaneously for fourteen days. All changes refer to the average value found in untreated animals weighing 240 gm. + 2 gm. Group II = controls, untreated. Groups V and VII = 12 units Protamine Zinc insulin daily; Group VIII = 120 units daily. All animals injected with cortisone received 5 mg./day. Many cortisone treated rats developed visible infections. Changes in these rats, and in a few others which did not gain weight, are shown in Groups VI B to VIII B.[20]

the prevailing one, at least in animals without severe infections.

Some animals received 120 units of insulin per day; the daily amount of cortisone remained the same. The insulin effect on adipose tissue was still more pronounced, while the effect on body protein was questionable. The increase of body fat in cortisone-insulin treated rats occurred rather independently from the gain of total body protein. Furthermore, such adipose tissue showed normal fat, protein and water content.[20]

In other rats, subjected to similar treatment for a variable period of time, hepatic and adipose tissue lipogenesis were compared (figure 4). The normally very low hepatic lipogenesis increased considerably when insulin was administered for four days before the experiment. This effect, however, was only temporary. After fourteen days the same normally low values were observed as in the controls.

Lipogenesis by adipose tissue remained accelerated during the entire period of insulin treatment. Cortisone diminished lipogenic activity in both hepatic and adipose tissues. When both hormones were administered simultaneously, the insulin effect was the prevailing one. But again, only adipose tissue lipogenesis remained markedly accelerated throughout the entire experimental period. Essentially the same results were observed by using 5 mg. of cortisone and 4 to 120 units of insulin.[5]

These experiments demonstrate that one of the predominant actions of insulin is that on adipose tissue. This

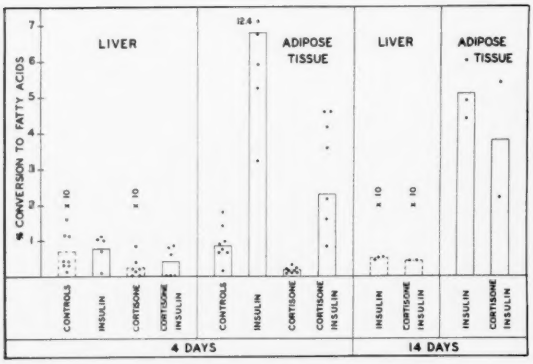

FIG. 4. In vitro lipogenesis by hepatic and adipose tissue of rats pre-treated with cortisone, 5 mg./day, and/or Protamine Zinc insulin 12 units/day, for four and fourteen days, respectively. Some values for hepatic lipogenesis have, for the purpose of demonstration, been multiplied by ten. The per cent conversion in these cases was uniformly smaller than 0.2 per cent. Pooled tissues of three rats were used in each experiment.[5]

TABLE 2

I. Obesity, islet hypertrophy and hyperadrenocorticism

a. Cushing's syndrome (Cushing[21])
b. Steroid diabetic guinea pigs[22]
c. Mice, bearing ACTH-secreting anterior pituitary tumors[23]
d. Mice, implanted with pellets of compound A or B[12]
e. Mice, castrated[12]
f. Mice, old[12]

e and f: adrenal cortical adenomas
d, e and f: marked response only in certain strains

II. Obesity and islet hypertrophy

a to f
g. Hereditarily hyperglycemic obese mice (Mayer et al.[24])
h. Obese humans (Ogilvie[25])

III. Obesity and hyperadrenocorticism

a to f
i. Mice, implanted with pellets of compound A or B (Kendall[26])
k. Certain forms of adiposity in children (Simpson et al.[27])

IV. Obesity without islet hypertrophy and without hyperadrenocorticism

l. Rats, hypothalamic obese[12]

V. Hyperadrenocorticism without obesity and without islet hypertrophy

m. Rats, (Wistar) implanted with pellets of compound A, B, E or F[12]
n. Rats, (Osborne-Mendel) bearing adrenal cortical adenomas (Mulay[28])

FIG. 5. Pancreas of a normal mouse of the LAF_1 strain, weighing 28 gm. An area with one large and one small islet is shown. Normally, most of the islets are of the small and middle size type. X100.

action remains unimpaired in the presence of an insulin antagonist like cortisone, which exerts its influence apparently more in other tissues. It should be mentioned here that incomplete experiments show that insulin also moderately enhances deposition of dietary fat, and markedly reduces the turnover of adipose tissue fat.

Hyperadrenocorticism frequently is associated with obesity and islet hypertrophy. Table 2 lists some forms of obesity, with and without known endocrinological disturbances.

Enlargement of the islets of Langerhans in Cushing's syndrome was first noted by Cushing himself.[21] We became interested in the possible etiological connection between islet hypertrophy and adiposity when we observed both features in steroid diabetic guinea pigs.[22,18] Subsequent search revealed islet hypertrophy in several other forms of obesity.

A few photomicrographs demonstrate extent of this enlargement. The pancreas of a normal mouse (LAF_1 strain) is shown in figure 5. An area with a relatively large islet was selected.

Figure 6 demonstrates islet hypertrophy in a severely obese mouse of the same strain, in which obesity was

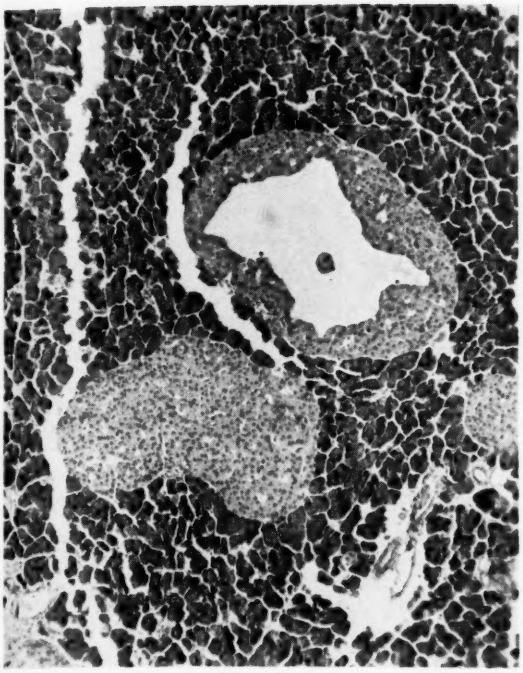

FIG. 6. Pancreas of a male mouse of the same strain, made obese by implanting ACTH-producing anterior pituitary tumor. The body weight increased within four months after the implantation from 23 gm. to 51 gm. Controls showed at the time of sacrifice an average body weight of 30 gm. The increase in total islet volume in tumor-bearing mice parallels closely the degree of obesity. The larger islets which are very numerous often show central cavitation. Many of the enlarged beta cells are partly degranulated.[23] X100.

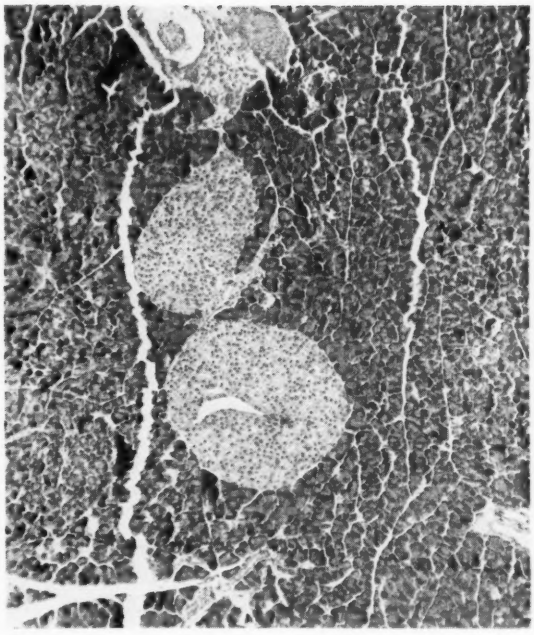

FIG. 7. Pancreas of a female mouse of the same strain, in which obesity was induced by implanting pellets of dehydrocorticosterone for thirty-three days. The amount of steroid absorbed per day was 1.2 mg. The experimental animal increased its body weight within this time from 22 gm. to 34 gm., controls to a maximum of 24 gm. The enlargement of the islets, although marked, is less pronounced than in the preceding slide, and so are cavitation and loss of beta cell granules.[12] X100

induced by implanting ACTH-secreting anterior pituitary tumor, kindly supplied by Dr. J. Furth, Boston. At the beginning of the experiment, the mouse weighed 21 gm. Four months later, at sacrifice, the body weight had increased to 51 gm. and the amount of extractable body lipids to 24.5 gm. Body weight and fat content of controls varied between 28 and 33 gm., and 3.1 and 4.2 gm., respectively.

Other mice of the same strain were made obese by using Kendall's[26] method of implanting pellets of Compounds A and B.* Figure 7 shows the pancreas of a mouse made moderately obese by implanting pellets of 11-dehydrocorticosterone (compound A) for thirty-three days. The controls increased their body weight from 21 gm. to a maximum of 24 gm. and showed at sacrifice a fat content of 2.2 gm.

The experimental animal reached a body weight of 34 gm. and the fat content at sacrifice was 14.7 gm.

Islet hypertrophy has been described by Mayer et al.[24] in hereditarily hyperglycemic obese mice, and by Ogilvie[25] in thirteen out of nineteen cases of human adiposity. Simpson and co-workers[26] found significantly increased urinary excretion of hydrocortisone in a certain type of obese children.

It is quite interesting to note that hyperadrenocorticism in rats does not induce adiposity as it does in mice. Neither does administration of Compound A, B, E or F induce obesity,[12] nor does the development of adrenal cortical tumors.[28] The islet hypertrophy after prolonged administration of these compounds, if present at all, is minor. In mice, however, administration of compound A and B produces adiposity, the extent of which varies considerably according to the strain employed, as demonstrated by Kendall. The same strains of mice which react favorably to compound A and B, fail to develop obesity if cortisone is administered.[29]

Obesity develops in certain strains of mice as the animals grow old, or after castration. Again, we noted

* Kindly supplied by The Upjohn Company.

hypertrophy of the adrenals and the islets of Langerhans.[12] On the other hand, Wistar rats, made severely obese by hypothalamic lesions, show no significant enlargement of the islets.[12] Assuming that our theory concerning the etiological mechanism in these forms of obesity is correct, one is tempted to speculate that the excess weight gain during or after the menopause is caused by a similar mechanism.

It is obvious from these observations that at least two major factors are responsible for the development of adiposity in hyperadrenocorticism, and probably in some other forms of obesity. One factor is the type of stimulus acting on the islets, and the second one the specific reaction of the islets, depending on species and strain.

Unfortunately, not much is known about the mechanism by which insulin secretion is governed. Still more limited is our knowledge of the regulation of adipose tissue metabolism and growth.

Hyperadrenocorticism apparently does not interfere with the action of insulin on adipose tissue. Other hormones, although they may directly or indirectly stimulate increased release of insulin, may at the same time counteract its effect on the fat cells.

SUMMARY

Insulin acts directly on adipose tissue, accelerating lipogenesis and growth which in turn leads to increased food intake. Cortisone and the adrenal cortex diminish lipogenesis. In the presence of sufficient amounts of insulin, however, the insulin effect on adipose tissue remains the prevailing one. Hyperadrenocorticism is frequently associated with obesity in certain species. Whenever marked obesity develops, hypertrophy of the islets can be observed, suggesting increased secretion of insulin. It should be noted that only certain corticosteroids induce obesity, and that the same steroid which produces marked adiposity and islet hypertrophy in one species may be without effect in the other one. Severely hypothalamic obese rats show no islet hypertrophy. These findings imply that adiposity seen in hyperadrenocorticism is the result of excess insulin release.

SUMMARIO IN INTERLINGUA

Le Action De Insulina E De Cortisona Super Histos Adipose

Insulina exerce un action directe super histos adipose. Illo accelera le lipogenese e le crescentia, e isto—de su parte—resulta in un augmento del ingestion de nutrimentos. Cortisona e le cortice adrenal reduce le lipogenese. Tamen, in le presentia de sufficiente quantitates de insulina, le effecto de insulina super le histos adipose remane intacte. Hyperadrenocorticismo es frequentemente associate con obesitate in certe species. Quandocunque marcate grados de obesitate se disveloppa, hypertrophia del insulas pote esser observate, e isto suggere un augmentate secretion de insulina. Il debe esser signalate que solmente certe corticosteroides es capace a inducer obesitate e, in plus, que un steroide identic pote producer marcate grados de adipositate e de hypertrophia del insulas in un specie e remaner sin effecto in un altere specie. Rattos obese que es severmente hypothalamic exhibi nulle hypertrophia del insulas. Iste constatationes indica que le adipositate que es vidite in casos de hyperadrenocorticismo es le resultato de un liberation excessive de insulina.

ACKNOWLEDGMENT

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DISCUSSION

WILLIAM C. STADIE, M.D.: I take this opportunity of saying a few words about the mechanism of insulin action in adipose tissue. These remarks are mostly of a speculative nature, but the evidence I show here might give us a starting point.

Table 1 shows unpublished data from our laboratory obtained by Haugaard and Marsh. They used adipose tissue in vitro in the presence of certain substrates and measured the effect of insulin upon oxygen uptake.

In the presence of insulin, there was consistently found—the figures represent the means of from ten to twenty determinations—an increase of oxygen uptake ranging from 11-27 per cent in the presence of glucose, succinate, lactate, pyruvate, and somewhat less than in the case of acetate.

An increase in oxygen uptake of tissues in vitro in the presence of insulin is rare. The diaphragm—this has been consistent in our experience and in the experience of others—does not respond to insulin with an increase in oxygen uptake, although it uniformly shows an increase in glucose uptake and carbon dioxide formation.

I might say that this same phenomenon of increased oxygen uptake by adipose tissue can be demonstrated in the presence of so-called bound insulin; that is, when adipose tissue is previously momentarily exposed to insulin, then washed and finally equilibrated with glucose without further addition of insulin to the medium, one observes an increase in oxygen uptake. The amount of insulin found in the adipose tissue is extremely small.

There is one tissue that has been shown to respond to insulin by an increased oxygen uptake originally reported by Krebs in pigeon muscle. But the increase in oxygen uptake is different from the type observed in adipose tissue. It is a slow increase, requiring some half-hour to an hour to manifest itself appreciably, as if some form of adaptive mechanism were in operation.

In the case of the adipose tissue, the response is immediate. What this means we do not know, so far as the mechanism of the action of insulin is concerned. We can clearly rule out an action on hexokinase because, with the exception of glucose, the substrates require no hexokinase for dissimilation.

Whether transport into the cell is acting here we do not know either, but I would be rather inclined to think that that is not the case because, with the exception of glucose, these substrates are, presumably, freely diffusible into the tissue cells. We are compelled to fall back, in a speculative way entirely, on Krebs' original idea, which he advanced when he observed this phenomenon in pigeon muscle, namely that insulin might accelerate some phosphorylation mechanism in the Krebs cycle, which when stimulated, increases oxygen uptake.

TABLE 1

Effect of insulin on oxygen uptake of retroperitoneal adipose tissue from normal rats
(Haugaard and Marsh)

Substrate	Insulin effect on oxygen uptake
None	+ 6 ± 5.0
Glucose	+ 22 ± 4.1
Succinate	+ 27 ± 4.5
Lactate	+ 25 ± 3.3
Pyruvate	+ 11 ± 4.5
Acetate	+ 8 ± 0.5

FRIEDRICH WASSERMANN, M.D.: I am very grateful for being given the opportunity to make, as an histologist, a few remarks and to raise a question in connection with Dr. Hausberger's presentation. Dr. Hausberger's results are related and give strong support to a concept of the origin, structure and function of adipose tissue, for which Dr. Hausberger and I are sharing the responsibility, since it was first published in 1926 (*Zeitschrift f. Zellforschung u. Mikr. Anat.* 3:235-328).

The then new concept of adipose tissue was based on the observation that in mammalian and human embryos, everywhere fat depots are later situated, little organs appear, called primitive organs of the fat lobules, in which after a while the cells accumulate fat. The primitive organs consist of a network of blood capillaries within a network of mesenchyme cells. The fact we are stressing here is, that without exception, where there are fat lobules later on, these little organs must be formed

beforehand. They are characterized by the intimate relationship between mesenchymal cells and capillaries. Therefore, they could be termed "reticulo-endothelial organs" and grouped together with blood-forming and lymphatic organs which have fundamentally the same structure.

This already points to the fact that adipose tissue is not just a modification of areolar connective tissue, as it was believed to be by the histologists, but a tissue, or better a system of specific organs, of high activity, instrumental in the synthesis and the release of fat.

How these primitive organs are formed could be shown in a situation very suitable for this purpose, viz., the appendices epiploicae of the colon in the newborn human. Although these peritoneal appendices are places where a great amount of fat is later accumulated, no fat is present in the connective tissue of the still very small appendices epiploicae at the time of birth, while in the subcutis and elsewhere in the body fat has been already deposited abundantly. Before this can happen in the appendices the organization which corresponds to the primitive organs of the embryo must be built. We observe the sprouting of new capillaries from the walls of the small blood vessels in the appendices shortly after birth and the simultaneous spreading of mesenchyme cells deriving from the adventitia of the same vessels.

The important point is that evidently a reaction of the mesenchyme, which is present at the walls of the small blood vessels, has to take place in order to develop the organization that brings about the differentiation of mesenchymal cells into fat cells.

This mesenchyme reaction is the basic process in the formation of adipose tissue under both normal and pathologic condition, as our extensive studies have demonstrated.

Now, Dr. Hausberger has shown today a remarkable difference between an ordinary adiposity due to hyperphagia and an insulin-produced obesity. The ordinary obesity—we may say a kind of passive obesity—is limited by a certain ceiling. This is not so in insulin-produced obesity. We can understand this. In the ordinary obesity the limited capacity of the fat cell seems to be responsible for the ceiling. But in the other case, as Hausberger has shown, mesenchyme cells, which are kept in reserve, so to speak, in the fat organs, become activated.

My question is, whether beyond this, when the mesenchyme reserves are exhausted, or at a certain intensity of the insulin effect, also the fundamental reaction of vascular mesenchyme, inside or outside of the existing fat organs, may be induced by insulin as a repetition of the embryonic process.

We do not know whether this may occur in extreme ordinary obesity, but we know that it occurs in the formation of lipomas. Many years ago, in Aschoff's Institute of Pathology a malignant lipoma was studied, which despite repeated resections continued to grow and finally depleted the body of its fat and proved to be fatal. Microscopic examination of this lipoma revealed that it was produced by the continuous growth of the mesenchyme of the vascular sheaths, that is by the same reaction of the vascular mesenchyme that marks the beginning of the formation of adipose tissue in the embryo.

My question is—and I believe it would be of great interest to know—whether or not insulin may act as a humoral agent that produces this mesenchyme reaction.

I may also remind you, in concluding, that insulin deficiency delays wound healing. In wound healing, a very similar mesenchyme reaction is involved in the production of granulation tissue. If we could avoid infection of experimental wounds in diabetic animals, we might be able to recognize whether insulin is a factor instrumental in this reaction.

ROBERT S. GORDON, JR., M.D.: I think adipose tissue, from the point of view of biochemical and physiological studies, is in the condition of Cinderella just before the fairy godmother arrived. The liver and skeletal muscle have long since been escorted to the ballroom, (the office of *JBC* and other leading science journals), whereas the poor adipose tissue has heretofore uniformly been left in the wastebasket. I think Dr. Hausberger and others who have spoken are to be congratulated for having been among the pioneers in investigating adipose tissue for its own sake to see if it might not be something of metabolic significance.

I wonder whether some of the delay in the study of adipose tissue may not have come, not only from the fact that the tissue is difficult to work with and poorly localized in the animal organism, but also from the fact that we have learned that animals, as a whole, obey the law of conservation of energy. Adipose tissue takes up the slack of caloric intake and output and, as a result, the reaction of adipose tissue to one or another experimental regimen can usually be predicted with accuracy. If you add more calories, the adipose tissue increases, and the opposite is true if you fast or give a low caloric intake to an animal. However, we still know little of the mechanism of such effects and the present study, related directly to the effect of hormonal agents on the tissue is, therefore, all the more significant.

I would like to recall for a moment some of the facts mentioned this morning. Dr. Dole's presentation brought up another mechanism whereby insulin may affect adipose tissue function. By a slightly indirect approach, he gave us evidence to indicate that insulin probably affects adipose tissue in such a way that its output of fatty acids, the lipolytic activity of the tissue, is decreased. So the same agent that increases lipogenesis will decrease lipolysis.

I would like to mention very briefly that recently, in experiments at the National Heart Institute, we have studied the lipolytic activity of isolated adipose tissue fragments, incubated in vitro in media of known composition. In this system, which is highly simplified and not confused by the presence of other organs, nerves, or outside influences, it has been possible to demonstrate a direct effect of insulin added to the medium in decreasing the output of nonesterified fatty acid from the surviving adipose tissue fragment. So the effect of insulin in increasing the body mass of adipose tissue, may be the result of two effects: an increase in lipogenesis and a simultaneous decrease in lipolysis, both of which may very well be primary effects of insulin on the adipose tissue, and not secondary to the effect of insulin in any other part of the body.

ALBERT E. RENOLD, M.D.: I would like to add a few observations which have been recently made by Dr. Albert Winegrad and myself in Dr. Thorn's laboratory and which are, I believe, pertinent to the subject under discussion. We have studied the effects of insulin added in vitro upon the metabolism of glucose and other substrates by the epididymal fat pad of the male rat. Our technic has differed somewhat from that described by Dr. Hausberger in that we have used in each experiment only tissue from the same animal, comparing the fat pad on one side with that on the other side, and in that we have carefully avoided any unnecessary handling or cooling of the tissues prior to incubation. We believe that these details are important and explain the much greater effects of insulin which we have obtained, when compared with the observations of other investigators. Under these conditions insulin added in vitro increases glucose metabolism by isolated adipose tissue, with regard to glucose uptake, with regard to the oxidation of C^{14}-labeled glucose to $C^{14}O_2$, and with regard to lipid synthesis from glucose carbon. These insulin effects are of considerable magnitude—of the order of 200 to 300 per cent of baseline activity—and can be detected within fifteen minutes of the addition of the hormone. Also, in our laboratory Dr. Donald Martin has recently demonstrated that significant insulin effects can be detected with insulin concentrations as low as 10 microunits (10^{-5} or 0.00001 units) per ml., i.e., concentrations well within the range postulated for biological fluids such as plasma.

Figure 1 summarizes our observations concerning the effects of insulin added in vitro upon lipogenesis from glucose (G), acetate (A) and pyruvate (P). The asterisk following the designation of a substrate (e.g., G*) indicates labeling of the substrate with C^{14}. The black column indicates the metabolic activity of the tissues incubated in the presence of insulin, the shaded column the activity of their paired controls incubated without insulin. It appears from these data that insulin stimulated lipogenesis from glucose, but not lipogenesis from acetate alone or pyruvate alone. However, when unlabeled glucose was added to labeled acetate or pyruvate clear-cut stimulation of lipogenesis from acetate and from pyruvate resulted from the further addition of insulin. These results suggest that increased glucose metabolism is a prerequisite for the stimulatory effect of insulin upon lipogenesis and thus fit the hypothesis of a single site of insulin action in this tissue, primarily leading to accelerated glucose uptake and/or phosphorylation.

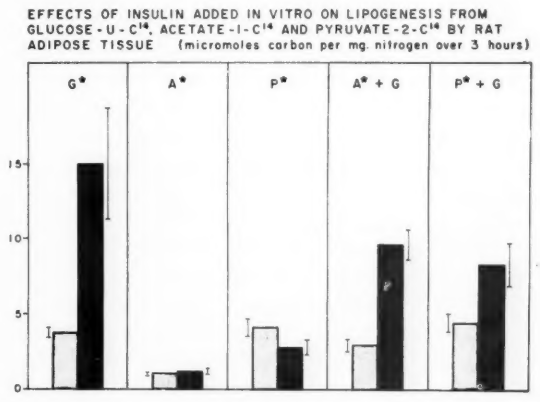

FIG. 1. Substrate concentrations used: Glucose-U-C^{14} (G*) 20 mM./liter, Acetate-1-C^{14} (A*) 60 mM./liter, Pyruvate-2-C^{14} (P*) 40 mM./liter, unlabeled Glucose (G) 10 mM./liter. Insulin concentration, when present, was 0.1 unit per ml. Height of columns indicates lipogenesis with (black columns) and without (shaded columns) insulin added in vitro; brackets indicate two standard errors of each mean.

We have also investigated the effects of insulin added in vitro upon the metabolism of carbon atoms one and six of glucose by this tissue and in so doing we have measured both the formation of $C^{14}O_2$ and the incor-

poration of C^{14} into fatty acids. The results are summarized in table 1 and have been interpreted as follows:

TABLE 1

Effects of insulin added in vitro on the metabolism of glucose-1-C^{14} and glucose-6-C^{14} by rat adipose tissue*

	Oxidation to CO_2 of fatty acid		synthesis from	
	Carbon 1	Carbon 6	Carbon 1	Carbon 6
Control	0.92†	0.24	0.19	0.38
+Insulin	6.36	0.33	3.06	6.31

*Expressed as micromoles of carbon atom 1 or 6 per mg. tissue nitrogen over a period of three hours.
†Each figure represents the mean of six observations.

Firstly, as previously suggested by Milstein (*Proc. Soc. Exp. Biol. & Med.* 92:632, 1956) it would appear that both a glycolytic (Embden-Meyerhof) and a non-glycolytic (presumably the phosphogluconate oxidate) pathway are operative in rat adipose tissue. Secondly, the data concerning the incorporation of carbon atoms one and six into fatty acids suggest that insulin added in vitro stimulates *both* pathways of glucose metabolism to an approximately equal extent, a finding consistent with a major effect of insulin limited to glucose transport or activation. However, the surprisingly marked discrepancy between the insulin effect upon oxidation of carbon atom one and that upon oxidation of carbon atom six of glucose to CO_2 leaves open the possibility of a second direct insulin effect upon the phosphogluconate oxidative pathway. Finally, the coupling in all of these experiments of greatly increased metabolism of glucose-6-phosphate by way of the TPN-dependent glucose-6-phosphate dehydrogenase with greatly increased lipogenesis agrees fully with the concepts so clearly discussed earlier by Dr. Siperstein, although relating to another tissue, liver.

In closing I would like to thank Dr. Hausberger for his insistence upon the metabolic importance of adipose tissue and also for having pointed with Dr. Milstein to the suitability of epididymal rat adipose tissue for studies in vitro.

SYDNEY S. LAZARUS, M.D.: I have read with interest much of Dr. Hausberger's work. Unfortunately, I find that in certain respects I may have to disagree with him, or perhaps there is some other interpretation of certain personal experiences with the effect of steroids in rabbits.

With cortisone and hydrocortisone it has been found that rabbits show a marked loss of body weight, which in six weeks may be as much as one third of the initial weight of the animal. This is accompanied by marked hyperplasia of the pancreatic islets, a moderate increase in mitotic activity, increased size, and degranulation of β cells. These morphologic alterations are considered to be, in this instance, indicative of increased functional activity of β cells with increased insulin output from the islets.[1-3] However, in this animal species there is a marked diminution in fat depots.

Perhaps Dr. Hausberger has an alternative explanation of the increase in fat depots which occurs in the guinea pig and the mouse under the influence of excessive dosages of adrenal steroids.

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F. X. HAUSBERGER, M.D.: First, I would like to answer Dr. Wassermann's questions.

I certainly think that insulin influences, or stimulates directly, the mesenchyme anlage for the adipose tissue and produces new fat cells. This occurs, apparently, within the single fat lobule, because the size of the lobule itself grows larger. I have not had the impression that the number of lobules increases but the number of cells within the lobule. I certainly think it is stimulation of the mesenchyme which is present here, causing it to be changed in a special way in order to produce fat cells.

With regard to the question by Dr. Lazarus, as to why rabbits do not get obese if one gives cortisone or corticosteroids: We tried different species ourselves, among them mice, hamsters, and rabbits. I had the impression that in rabbits, in a rather early stage, three or five days after administration of cortisone, there is an increase in weight. We have investigated the adipose tissue. The changes are not significant, but they indicate an increase in weight of adipose tissue mass. After a short while these animals become susceptible to infections if one continues with cortisone administration. We have observed the same susceptibility in hamsters and mice and, to a much lesser degree, in guinea pigs.

There may be another mechanism which I am not able to explain, but I think that would be, for the time being, a suitable explanation.

Abnormal Fat Transport

W. Stanley Hartroft, M.D., Ph.D., St. Louis

INTRODUCTION

The aspects of abnormal fat transport to be discussed more nearly correctly come under the heading "pathologic effects of abnormal fat transport" for reasons to be mentioned. Fat is, of course, transported from one portion of the body to another mostly in the circulating plasma, and that fat which is attached to erythrocytes and leukocytes probably will not fall in the realm of this discussion. Because plasma can readily be withdrawn during life and placed in a test tube, it has been studied extensively by biochemists and biophysicists. But, because of its fluid property, it has not lent itself to more than simple gross and microscopic or electron-microscopic studies even in its coagulated stage. Therefore, there is little to say about the anatomic pathology of plasma in the transport of fat or any of its other elements. Instead, the topic will be discussed from the point of view of the anatomic pathologist around the possible pathologic *effects* of abnormal fat transport, presumed or otherwise.

If the only effects of abnormal fat transport were to be reflected in changes in plasma color, turbidity and viscosity, without associated structural and functional alterations in vessels that carry it, or organs supplied by it, the entire subject would be of little more than academic interest to those in the health professions. But abnormalities in fat transport by the plasma indeed almost surely are reflected by a number of serious structural and functional changes in body tissues, and the latter in turn are matters of immediacy and even urgency to all engaged in the health professions. Despite the fact that this opinion may be widely shared, it is probably fair to state that the existence of a pathogenic link between more than a few recognized pathologic lesions and abnormal fat transport is hypothetical at this stage of knowledge. Therefore, such controversial possibilities as intercapillary glomerulosclerosis, diabetic retinopathy, atheroma, and myocardial infarction will be included in this discussion. First will be discussed the more generally accepted biophysical and biochemical patterns of fat transport and factors that influence them; secondly, a consideration of the diseases already mentioned. This sequence will permit a consideration of lesions—the anatomic lesions—presumed to be associated with abnormal fat transport.

Circulating plasma is the only major vehicle for transport of fat from one organ to another. This statement, however, has one important exception in the case of transport of absorbed fat from intestine to liver. Here, the major route is the lymphatic system and hence into systemic blood, except for fatty acids, twelve carbon atoms or less in length, which enter directly into the portal circulation.[1] Their absorption does not appear to be under the control of the adrenals,[2] since adrenalectomy depresses absorption of long-chain fats but not of tributyrin.

Before touching on biochemical complexities of fat transport, it will be helpful here to review a few generalities. The major, and probably only, purpose of moving fat from one part of the body to another, is either to supply immediate energy-needs of particular organs (by oxidation of fatty acids) or to store it in adipose tissue depots for their future needs. If fat enters blood from either ingested food or fat depots, it does not accumulate in serum beyond a certain level, but instead promptly enters parenchymal cells for utilization or storage. Any factor (hormonal, dietary, enzymatic, or even mechanical) that accelerates entrance of fat into blood, that inhibits its passage from plasma into cells, or that does both, will surely raise plasma lipid levels. Ill effects from such abnormality (hyperlipemia) may initially and most severely affect blood itself, perhaps producing important changes in coagulability, or the walls of blood vessels carrying such pathologic sera. Here, of course, atheroma comes to mind. And the naive concept, that plasma lipid which can not be deposited in normal sites (parenchyma or adipose tissue) must perforce precipitate on adjacent red blood cells and vessel walls causing atheroma, may still be a useful working hypothesis. The foregoing is but a statement of truisms, justified only by fear that some of us may become so involved with complex minutiae that we lose sight of the obvious.

The late Dr. Harry J. Deuel stated,[3] "All types of lipids which are present in the tissues of animals occur

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in greater or lesser amounts in the blood," and, with one possible exception, mentioned later, this appears now to be true. Blood lipids consist of fatty acids of various chain lengths, neutral fats, phospholipids, and unsaponifiable components—cholesterol, carotenoids and the fat soluble vitamins. Carotenoids and fat soluble vitamins lie outside this discussion. Fatty acids exist in blood in combination with choline as phospholipids and they are relatively unsaturated.[4,5] The most unsaturated fatty acids in the blood are combined with cholesterol as esters, while the most saturated are carried as neutral fat in lipoprotein complexes.[6] Data of various investigators indicate that 35 per cent of the fatty acids occur as phospholipid, 45 per cent as triglyceride, 15 per cent as cholesterol esters, and the 5 per cent remaining are bound to albumin. Cholesterol, of considerable fashionable interest, is present in both plasma and red blood cells.[7]

During the latter part of this decade it has become increasingly clear from the work of a number of investigators, stimulated and led by the Gofman group,[8] that, for practical purposes, all plasma lipids circulate in the form of complexes with proteins rather than as free fat. The physical nature of these lipoproteins varies in both physiologic and pathologic states. In some instances, for example, renal and hepatic disease, lesions in the organs probably are the cause of changes in the physical characteristics of lipoproteins. In other cases, most notably atheroma and heart disease, alterations of lipoproteins have been considered a pathogenic step in the production of the disease. Here lies the greatest need for additional and clearer evidence. Lipoproteins of low density (S_f 40,000) can be seen under the microscope as chylomicra. Gofman's concepts[9] can be correlated with the microscopic appearance. Chylomicra can be seen by light microscopy as sudanophilic droplets enmeshed in coagulated plasma in vessels. In electron micrographs they appear as larger, osmophilic, high density dots. As various biochemical fractions of lipids rise in plasma, so usually do the larger, low density lipoproteins (S_f 40,000) as do the chylomicra seen with the dark-field microscope along with intensity of sudanophilia in coagulated plasma in frozen sections of fixed tissue and osmophilia of plasma in electron-micrographs.

At the present stage of our knowledge, the pathologist begins where the biophysicist leaves off: at the chylomicron level. A brief consideration of fat embolism will open the discussion. The traumatic or "surgical" type is familiar to all, and although not part of diabetes, it may serve as a model from which to proceed to a concept that might be called "medical" or "intermittent" fat thrombo-embolism. The second type may have some relation to diabetes (particularly to intercapillary glomerulosclerosis of Kimmelstiel and Wilson, and to diabetic retinopathy). Each subject becomes more speculative in sequence. Diabetic retinopathy will permit introduction of atheroma and myocardial infarction.

TRAUMATIC FAT EMBOLISM

Since consensus states that traumatic fat embolism is the most frequent cause of death following fracture of long bones,[10] it becomes of more than academic importance. Traumatic fat embolism is defined as fat in circulating blood in the form of globules sufficiently large to obstruct arterioles and capillaries rather than in the form of a fine emulsion of a metabolic lipemia as numerous chylomicra or lipoproteins of S_f 40,000 or more. The globules obstruct small arterioles and capillaries of lungs, kidneys, and brain. Death is accompanied by dyspnea with mental confusion, oliguria, and, when looked for, lipuria. Fat drops present in urine are diagnostic. In addition, hemorrhages of retinal fundi may be discovered, produced by fat emboli that have plugged capillaries. Contrary to earlier hypotheses, quantitative estimations of the amount of fat present in long bones do establish that there is plenty in the bone to account for the amount found in embolic sites.[11] Most fat in emboli is triglyceride, and Peltier, whose work I have been quoting, has found that fatty acids are more toxic than neutral fat. He does not advise using heparin or lipoprotein lipase in the treatment of fat embolism, as it would enhance breakdown of neutral fat to more toxic fatty acids.[10,12] As there is enough fat in bones to account for the total amount in embolic sites, there is no need to implicate consequent disturbances of the physical-chemical equilibrium of fat in plasma of patients suffering fat embolism. Such concepts only cloud the issue. Pathologic lesions in fat embolism are simple: Affected vessels are plugged with fat, and, in lungs and brains at least, small areas of local infarction can be demonstrated. In the kidney, fat emboli are restricted almost entirely to glomerular capillaries, and few other pathologic effects are seen. Shock is the usual cause of death, with acute failure of the right ventricle produced by mechanical obstruction of pulmonary vessels by emboli. In patients who survive longer periods, an unexplained but precipitous drop in hemoglobin and a rapid development of anemia are frequent.[14] This process may be associated with pulmonic hemorrhage.[15] Patients who recover from fat embolism rarely suffer irreversible renal damage or impairment of function.[16] Fat embolism following fractures of long bones

has a special predilection to become clinically significant in alcoholics.

INTERMITTENT FAT EMBOLISM

Traumatic fat embolism serves to introduce the next less well-recognized form of abnormal fat transport. This type has been referred to as medical fat embolism, or chronic intermittent fat embolism, to distinguish it from the single catastrophic shower of large emboli associated with fractures. This concept first arose in studying organs of rats that had been subjected to prolonged periods of dietary lipotropic deficiency, and in which fatty and cirrhotic livers had developed. Intravascular globules of fat were found in septal arterioles and capillaries of lungs and in preglomerular and glomerular vessels of kidneys. Although only small focal lesions (hemorrhage or edema) were noted in the lung, in the kidney the emboli had produced focal glomerular necrosis which in survivors later assumed some of the morphologic characters of focal glomerular sclerosis (Kimmelstiel-Wilson disease). The suspicion that intravascular fat globules had originated from ruptured fatty cysts in livers of these animals was strengthened because a few emboli consisted of ceroid. It is the alcohol-insoluble sudanophilic pigment formed in fibrous trabeculae and cells of livers of these rats.[17] These observations led to further studies in man.[18-21] Previously, an association between fatty liver and sudden death[22] and between cirrhosis and intercapillary glomerulonephritis[23] had been recognized, although in neither instance had the possibility of fat plugs in vessels of lungs, or kidneys, respectively, been considered in the pathogeneses. The late Dr. Stanley Durlacher and co-workers[20] studied a series of twenty-five cases of sudden death in alcoholics with fatty livers. Five of the deaths were attributed to massive pulmonary fat embolism probably originating from the fatty livers (figure 1). More recently, Fadel and Sullivan[21] have reported another case of fatty liver and fat embolism in a forty-seven-year old alcoholic male in which many emboli were found not only in pulmonic septal capillaries, but also in glomerular vessels. Kent[19] found fat emboli in lungs of fifty-three diabetics who had not been subjected to trauma. In three instances almost every low power microscopic field contained them, and in more cases less extensive involvement was discovered. But he did not believe that fat emboli were clinically significant. In our investigations,[18] stimulated by the morphologic resemblance of lesions in kidneys of choline-deficient rats to those of diabetic patients with Kimmelstiel-Wilson disease, we found lipid plugs in all but four out of sixteen of these patients (figure 2). Although the term "fat embolism" was used to describe the plugs, subsequent reflection cautions that the terms "fat thrombi" or "lipid plugs" would probably have been more wisely chosen since sources of emboli in livers or other organs were not demonstrated. In some glomerular capillaries and other vessels of these patients, coagulated plasma in sections appears intensely sudanophilic, and enmeshed in it are numerous tiny fat droplets. By filtration, up to 20 per cent of the plasma is removed as the blood passes through the glomeruli. In a hyperlipemic diabetic perhaps the resulting degree of concentration within the glomerular capillary loops is sufficient to produce inspissation in situ of microscopic chylomicra as fat thrombi or plugs. At any rate, no other mechanism has been suggested for their formation. From animal experiments and the clinical evidence cited above, a concept is gradually arising that obstruction of small blood vessels by plugs of fat (embolic or precipitated in situ) may develop in certain conditions, notably alcoholism and diabetes. The existence of these lipid plugs has been demonstrated in man as well as in animals. Their significance—whether they may produce sudden death or irreversible renal and ocular lesions—is a matter that must await further evidence. The fact that traumatic fat embolism is clinically more severe in alcoholics with fatty livers may imply that here the acute condition has been superimposed on the chronic.

DIABETIC RETINOPATHY

In the diabetic, a second situation in retinal capillaries, mentioned above, exists where lipid emboli or plugs may act as pathogenic factors. Resemblance of certain features of retinal aneurysms in diabetics to those of glomerular sclerosis in Kimmelstiel-Wilson disease has suggested to some investigators a common pathogenesis. Eyes of the choline-deficient animals referred to above were not examined, and data from this type of experiment are not available. But Dr. Charles H. Pope,[24] in our laboratory, has, by special technics, confirmed that fat is deposited in walls of diabetic retinal aneurysms. He has evolved a concept of their pathogenesis in which transient obstruction of the vessels by plugs of fat may be the initiating event. We think that similar studies of cortisone-induced retinal lesions in rabbits[25] might also be rewarding.

ATHEROMA AND CORONARY OCCLUSION

Abnormal fat transport is notoriously suspect as the culprit in the pathogenesis of atheroma and coronary occlusion with myocardial infarction in man. The mechanisms invoked do not consist of simple obstruction of

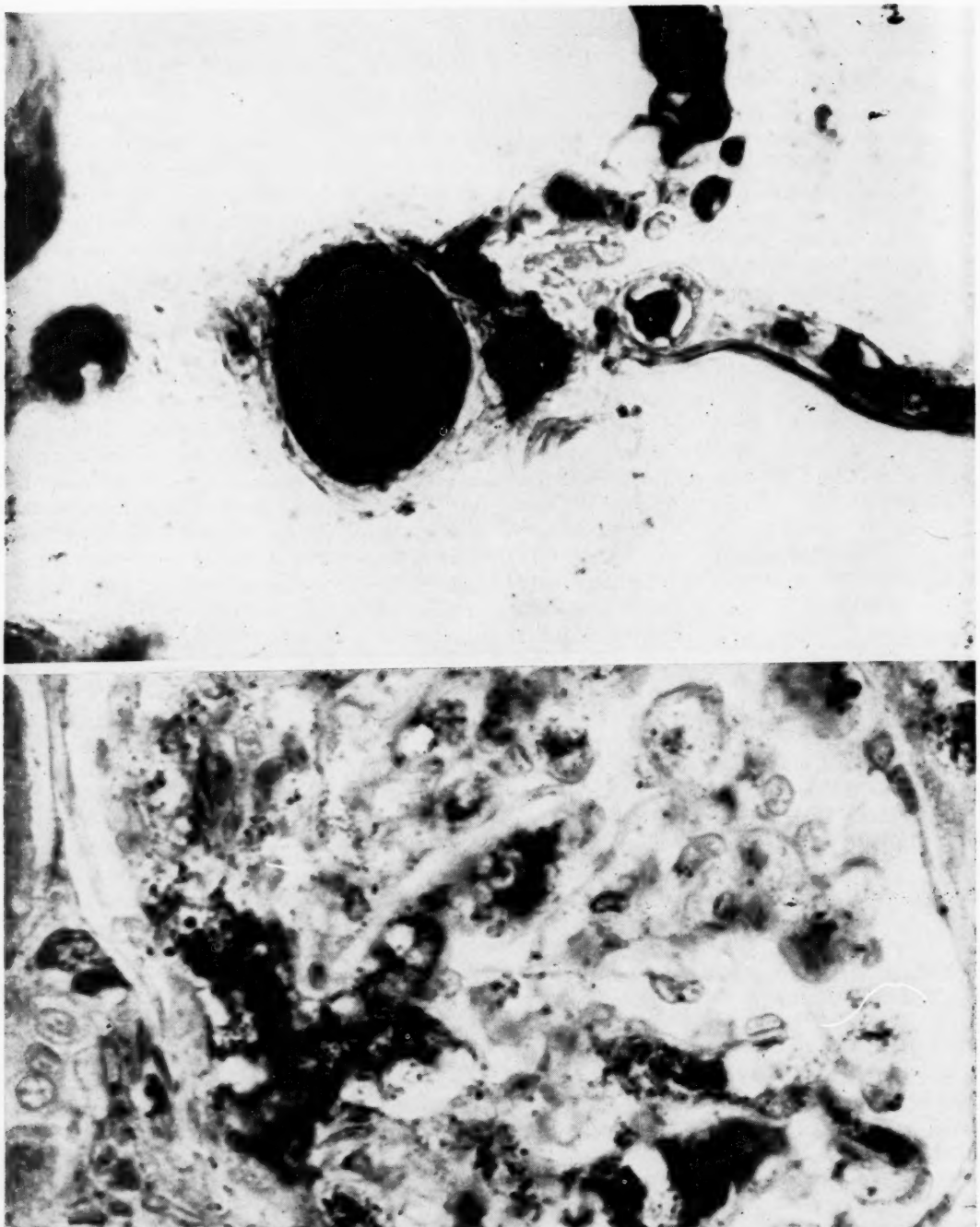

FIG. 1. (Top.) Fat embolism in a small arteriole and septal capillaries of the lung in a patient dying of acute alcoholism and severely fatty liver. The fat (black) appears solid and homogenous, completely filling the lumina. The nature of these fat emboli is to be contrasted with that of the lipid precipitates shown in figure 2. Frozen section, hematoxylin and Oil Red O. Approximate magnification X 800.

FIG. 2. (Bottom.) Precipitates of fat within glomerular capillaries have formed lipid plugs (black) filling and obstructing their lumina. Their granular nature, in distinction to the homogenous appearance of the emboli shown in figure 1, is apparent in the photomicrograph. Frozen section of the kidney of an adult diabetic, hematoxylin and Oil Red O. Approximate magnification X 800.

vascular lumina by plugs of fat, but instead are concerned with formation of atheromatous plaques and fatal coronary thrombosis. The brilliant contributions of Gofman, Keyes, Katz and others, have gone far to implicate lipoproteins and cholesterol of serum in pathogenesis of atheroma in man. Acting partly on their assumption that states of hyperlipemia and hypercholesterolemia are indeed atherogenic, we have attempted to push the production of experimental atheroma to its presumed "end product" of myocardial infarction in animals—an objective rarely before achieved. As a degree of success has already been reported,[26] the steps leading to these experiments and a brief resume of their results will be outlined. This is surely not out of order here since myocardial infarction occurs frequently in diabetics and is the commonest cause of their death.

My colleagues, Dr. W. A. Thomas, Dr. R. M. O'Neal and their associates, had been impressed for several years by the possibility that atheromatous plaques, in some situations at least, may in fact originate as compressed, organized thrombi that had adhered to the intimal lining. This theory is also currently and vigorously propounded by Duguid and his group.[27] It originated a century ago with Rokitansky but has been overshadowed by Virchow's imbibition theory involving cholesterol. Clark, Graef and Chasis[28] reported more than two decades ago that the so-called "fibrinoid" component of aortic and coronary atheroma in man is indistinguishable from the fibrinous component of thrombi. In studying the pulmonary atheromata in rabbits that follows repeated injections of blood clots into ear veins, Thomas discovered that high-fat meals concurrently stomach-fed to them intensified the size and number of the injected clots that survived intravascular lysis and ended as pulmonic arterial plaques.[29] Under the microscope he watched injected thrombi in vessels of living rabbit ear-chambers and was struck by the manner in which most clots were rapidly dissolved in the flowing blood, although a much smaller number resisted the lytic forces and presumably could have survived to become transformed into organized mural plaques. The enzymatic system in blood responsible for clot-lysis therefore appeared to constitute a practical and vital defense of the body against the many grave sequelae of intravascular thromboses and occlusions.

Stimulated by the inhibiting effect of high fat meals on clot-lysis that he had already demonstrated in living rabbits, he next studied the effect of orally administered fat on clot-lysing power of blood in vitro. Using streptokinase to activate lysis in the test tube, he discovered that clots of blood, drawn from rabbits three hours after they had been stomach-fed a single high fat meal, dissolved more slowly than similarly observed clots obtained from controls fed isocaloric amounts of sugar or equal volumes of water. Further, he found that meals of saturated fats, particularly butter, were more effective in prolonging streptokinase-clot-lysis time of the rabbit than was corn oil under identical conditions. Simplifying his system still further, adding directly small amounts of fat to a test tube of human blood and allowing it to clot, similarly prolonged its lysis time. Again, in these entirely in vitro experiments, butter was more effective than corn oil. Evidence from these three approaches—entirely in vivo, partly in vivo and partly in vitro and entirely in vitro—all yielded the same conclusion, namely, that fat, particularly some saturated fats, could inhibit clot-lysis and thereby favor persistence and organization of thrombi and presumably any sequelae arising from this abnormality, including vascular occlusion. It seemed to us that if some of the already familiar technics for producing atheroma in experimental animals were intensified in this direction by combining them with high levels of saturated fat in the diet, then clot-lysis might be sufficiently inhibited to favor thrombotic occlusion of coronary arteries so that actual myocardial infarction might result. Therefore, several different groups of rats were fed the diets shown in table 1. A high level of butter (60 per cent in terms of calories) was fed with supplements of thiouracil, cholesterol and cholic acid to induce hyperlipemia and hypercholesterolemia. A number of myocardial and renal infarcts developed in these animals, as reported in the table. They exhibited all morphologic features of myocardial and renal infarction encountered daily in our autopsy room at Barnes Hospital. Infarcts in the rats followed occlusion of nutrient arteries, and thrombi had formed in vessels in which the walls were loaded with fat but in which plaques were absent. Duration of the experiments was less than four months. Results suggested to us that fat in the walls had acted

TABLE 1
Ingredients in diet (percentage by weight)

	Groups						
	I	II	III	IV	V	VI	VII
Casein	20.0	20.0	20.0	20.0	20.0	6.0	6.0
Alpha soya protein	0	0	0	0	0	6.0	6.0
Sucrose	50.5	50.7	50.7	20.5	20.5	27.7	28.7
Salt mix*	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Alphancel	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Vitamin mix†	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Thiouracil	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Choline chloride	0.2	0	1.0	0.2	0.2	1.0	0
Sodium cholate	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Corn oil	10.0	10.0	10.0	20.0	0	0	0
Butter	0	0	0	0	40.0	0	0
Lard	0	0	0	0	0	40.0	40.0
Crisco	0	0	0	20.0	0	0	0
Cholesterol	5.0	5.0	5.0	5.0	5.0	5.0	5.0

* This salt mixture is the Wesson modification of Osborne and Mendel salt mixture. Science *75*:339, 1932.
† Each kilogram of the vitamin mixture contained the following triturated in dextrose:

	Grams		Grams
Vitamin A concentrate (200,000 units per gram)	4.50	Niacin	4.50
Vitamin D concentrate (400,000 units per gram)	0.25	Riboflavin	1.00
Alpha tocaphenol	5.00	Pyridoxine (hydrochloride)	1.00
Ascorbic acid	45.00	Thiamine (hydrochloride)	1.00
Inositol	5.00	Calcium panthothenate	3.00
Menadione	2.25	Biotin	0.02
P aminobenzoic acid	5.00	Folic acid	0.09

Incidence of myocardial and renal infarcts

Groups of rats*	No. of rats surviving 1 month	Rats surviving to end†	Average chol. levels‡	No. with myocardial infarcts	No. with renal infarcts	No. with either or both
I	9	8	1,225(7)	0	0	0
II	9	5	830(4)	0	0	0
III	10	7	2,430(5)	2	2	4 (40%)
IV	9	5	980(3)	0	0	0
V	10	2	3,360(2)	4	3	6 (60%)
VI	7	0	—	3	2	4 (40%)
VII	3	0	—	1	0	1 (10%)
Totals	57	27	—	10	7	15

* Initially, each group contained ten male rats.
† All surviving rats were killed after 131 days on specified diets.
‡ Cholesterol levels in the plasma were determined at the end of 131 days on the number of rats indicated in parentheses.

as a locus for formation and persistence of clots with occlusion of the lumen. These effects are surely related to gross abnormalities in plasma lipids induced by our deliberately unphysiologic dietary regimen. We have not controlled a large number of variables inherent in these experiments, but are now proceeding to do so. We hope, thereby, soon to extend our knowledge. We already know that thiouracil is necessary in this experimental regimen, and that corn oil and some hydrogenated fats have a protective effect. On the other hand, cod liver oil—also highly unsaturated—in other similar experiments did not protect as did the corn oil. We have confirmed our original experiments in a second series, and are starting to study effects of age and sex with this model.

Enmeshed in thrombi that formed in these rats, stainable fat including ceroid was discovered. Ceroid is probably the only lipid, albeit an abnormal one, found

in tissues but not in blood. These clots perhaps might be described more precisely by the term "lipocellular thrombi" or "plugs" to indicate the important role we believe the state of abnormal fat transport induced by our dietary regimen had played in bringing about their resistance to lysis and the resultant vascular occlusion. To this extent they may be thought of as a variant of the purer fat plugs and emboli previously discussed and included here for this reason.

CONCLUSION

Abnormal fat transport is obviously the pathogenic event in acute or chronic fat embolism. Its implication is not equally clear in some of the degenerative vascular diseases (atheroma, myocardial infarction, diabetic retinopathy). Evidence has been presented suggesting that a relationship may exist, but further investigation is needed to clarify the truth or falsity of this hypothesis.

SUMMARIO IN INTERLINGUA

Transporto Anormal De Grassia

Es obvie que le transporto anormal de grassia es le evenimento pathogene in acute o chronic embolismo de grassia. Non equalmente clar es le importantia de ille evenimento in varie morbos vaso-degeneratori. Es presentate datos que suggere que il existe un relation inter tal morbos e anormalitate del transporto de grassia, sed investigationes additional es requirite pro clarificar le validitate o le falsitate de iste suggestion.

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DISCUSSION

BRUNO W. VOLK, M.D.: Up to the last decade, the bulk of the work dealing with arteriosclerosis concerned itself primarily with problems in the field of biochemistry, physical chemistry, and pathophysiology. Only a comparatively small number of investigators have undertaken to study the basic anatomic changes underlying this disease, namely, those of the arterial wall.

The work of Dr. Hartroft and his colleagues, therefore, is a most welcome and interesting contribution to our knowledge of this so far relatively little understood complex disease. Since time does not permit me to go into too many details of his excellent presentation, I should rather like to confine myself to discussing several points which appear to be of particular interest to me.

Although spontaneous myocardial infarction has previously been stated to occur occasionally in rats subjected to various regimes, Dr. Hartroft appears to have succeeded for the first time in producing it in a significant percentage of these animals fed diets high in cholesterol and saturated fats. His work seems to contribute a very useful experimental tool for studying the effect of various dietary conditions in conjunction with other factors, such as sex, age, or diabetes, on the incidence and on the development of arteriosclerosis. The rat, furthermore, lends itself as an excellent experimental animal for the study of this disease, because of the similarity of its nutritional requirements to those of man, because of its small size, its short life span, and also because of the sex difference in regard to susceptibility of this animal to the development of fatty lesions in the coronary arteries resembling those of the human.

As Dr. Hartroft has previously mentioned, several authors, notably Wartman, Thomas, Duguid and others, have demonstrated that organized thrombi can be transformed into atheromata. In view of his discussion, it seems rather intriguing to speculate to what extent certain nutritional factors may have contributed in these experiments to the inhibition of clot-lysis. It might, furthermore, be interesting to conjecture whether, in these studies, the fats liberated from the lipid capsule of the red blood cell within the clot may have contributed to the atherogenic process.

Dr. Hartroft demonstrated thrombi within the renal arterioles of his animals and thus advanced a very interesting new concept concerning the pathogenesis of Kimmelstiel-Wilson disease that is somewhat at variance with what we are used to thinking of in this connection, particularly in view of the studies of others which have suggested that this lesion is primarily based on the thickening of the basement membrane of the capillaries in the renal glomeruli. I wonder whether Dr. Hartroft can tell us in how many patients with Kimmelstiel-Wilson disease he observed fat emboli and thrombi in the glomerular capillaries.

He also demonstrated the very frequent occurrence of renal infarcts in his rats. I wonder whether these infarcts are secondary to the thrombi in the heart or whether they were due to changes in the arterial wall itself. Atherosclerosis in the renal arteries is rather uncommon under experimental conditions. This leads us to speculate why certain arteries are more susceptible to atherosclerosis than others.

Holman and his associates have recently attempted to stain vitally with Evans Blue dye, certain necrotizing lesions in large arteries of dogs which have been subjected to a combination of high-fat diets and renal insufficiency. They observed that not only did the early lesions stain quite intensely with the dye but that in many instances did the site of predilection for those lesions stain even in normal dogs, although no histologic changes could be made out in the arterial wall.

Coming back to arteriosclerosis, it might very well be that in addition to certain anatomic peculiarities of the arterial wall such as, for instance, the avascularity of the intima, or certain changes of hemodynamics within the blood vessels, that certain physico-chemical differences or alterations in the ground substance may present contributory factors to abnormal transport and deposition of fat in the arterial wall.

WILLIAM S. COLLENS, M.D.: Dr. Hartroft's attempt to recreate the picture of a Kimmelstiel-Wilson lesion by means of fat embolism is intriguing and thought-stimulating.

I should like to call attention to an observation we made and published in a study of diabetics whose serum we subjected to analysis in Gofman's laboratory for the concentration of S_f 12-20 and 20-100 fractions of lipoproteins. We found that diabetics who presented clinical manifestations of a Kimmelstiel-Wilson syndrome had a much higher concentration of S_f 12-20 and 20-100 group of lipoproteins than those diabetics who did not have any renal complications.

HAROLD RIFKIN, M.D.: I should like to ask Dr. Hartroft how the lesions in these choline-deficient animals compare with the glomerular lesions induced by various adrenal steroid hormones. Also, I should like to ask whether urines of the choline-deficient animals were examined for doubly refractile fat.

DR. HARTROFT: I would like to thank all the commentators very much. In reply to Dr. Volk's question:

When we published our study of patients with Kimmelstiel-Wilson disease, we demonstrated intravascular fat plugs—they should not have been called emboli in these patients with Kimmelstiel-Wilson disease—in twelve out of sixteen cases. Since then we have extended the study and found this percentage to fall between 60 and 70 per cent. The fact that it is absent in 30 to 40 per cent may be explained by the stage of the disease. If you don't see it in the active progressive stage, you may not find the lipid plugs.

I know of your work, Dr. Renold. I am very sorry I didn't cite it. It is strong support for this theory. Thank you very much for bringing it out.

Dr. Rifkin, I have not studied the cortisone-induced lesions in the kidney, but I have studied the cortisone-induced lesions in the eye—and those, of course, resemble cortisone-induced lesions in the kidney. Dr. Bernard Becker discovered this lesion first in rabbits. A young man who is now in the Army but is coming back with me, Dr. Charles Pope, has this problem as a project. In man, he has observed deposits of fat in what he thinks are the lumina of the small retinal vessels. He believes it produces bulging of the walls, which then leads to the aneurysm-formation.

Why only the renal arteries and the coronary arteries were involved in our rats is difficult to answer. I would have expected to see infarcts in brain as well, and perhaps we still will. None was present in spleen. I can only attempt to relate this to rate of blood flow of the various organs.

The whole matter of the correlation in man of vascular involvement of various organs is practically unknown, in my opinion, at this time. We do not know whether atheromata in the coronary artery truly parallel the development of atheromata in the aorta. I am happy to say that pathologists all over the world are now taking this as a major project, and the answer will be forthcoming in two or three years.

I have just come from a conference sponsored by the World Health Organization, in Washington, which went on all week, and at which there were more than twenty pathologists from all over the world. We have formulated very definite criteria and technics to obtain these data from autopsy material and to answer something on which we acknowledge we have been slow to give you the answer.

The Duty of the Researcher

Human research resembles yet differs from general practice and requires special legal and ethical standards. The legal doctrines of malpractice carry over in principle to the conduct of medical research. The base of malpractice is found in the relation between physician and patient. Generally speaking, it covers any conduct on the part of a physician that does not conform to good or standard medical practice, resulting in injury. His failure to meet the standards may be due to ignorance or lack of skill, willful departure from accepted practice, negligence or breach of positive law, such as failure to obtain consent. These are strictly imposed since the physician deals with the most sacred of human values.

This norm seems to apply equally to the research scientist who does not live up to the controlling standards for the profession of scientific investigation. Such standards are undoubtedly more difficult to define, since the essence of research is untried and novel procedure. They therefore partake more of "how" than "what." The physician in regular practice may reasonably be tested by his use of accepted measures in specific situations. In research, standards must relate to how the investigator proceeded and how he checked himself.

The means by which research of high quality has been managed and the safeguards employed to protect the subject can be generalized into a set of precepts similar to that governing malpractice, to serve as a guide. Thus, willful or negligent deviation, resulting in injury, would constitute a basis for liability. On the other hand, observance of all known precautions, even in the event of an untoward result, would protect the honest, qualified investigator. Neither he nor the physician is a guarantor of success, but each has a responsibility to his own law and credo.

From "Human Experimentation," by Irving Ladimer, J.D., in *The New England Journal of Medicine,* July 4, 1957, pp. 18-24.

The Nature and Correction of Diabetic Ketoacidosis

William H. Daughaday, M.D., St. Louis

The arrival on a hospital floor of a comatose, profoundly dehydrated, and deeply breathing diabetic patient is a challenge to the knowledge and therapeutic skill of any physician. If the patient is untreated, death will not be long delayed. It may still occur if treatment is planned on inadequate clinical or laboratory examinations, or is done in a fumbling and vacillating manner.

The principles of the early treatment of diabetic acidosis which I consider of prime importance are summarized in table 1. Insulin rightfully heads the list because the absolute or relative lack of insulin is the basic deficiency of diabetic acidosis. Refractoriness to insulin exists in nearly all patients in diabetic acidosis. Acidosis and its associated electrolyte disturbances contribute to this critical state. Also, the presence of a serum protein inhibitor of insulin is a factor.[1] Diabetic acidosis is a stimulus for adrenal cortical hyperactivity which further antagonizes insulin action. Although it has been difficult to demonstrate a clear advantage of insulin doses greater than 40 units per hour in most patients with diabetic acidosis,[2] certain patients undoubtedly require truly massive doses of insulin to restore carbohydrate metabolism.

An initial dose of 200 to 300 units of insulin is recommended for profoundly acidotic patients. Part of this insulin may be administered intravenously. Intravenous therapy is particularly important in patients with circulatory collapse, which prevents adequate absorption of insulin from subcutaneous deposits. Insulin administration is pushed vigorously with doses of 50 to 100 units per hour until a significant decrease in blood sugar or glycosuria becomes evident. This break in hyperglycemia is a reassuring prognostic feature because it indicates that the patient is not one of the relatively rare cases in whom truly massive doses of insulin are necessary. In mild ketosis large doses of insulin are unnecessary and may provoke dangerous hypoglycemia.

Presented at the Symposium on Fat and Diabetes sponsored by The Clinical Society of the New York Diabetes Association, Inc., on Oct. 12, 1957.

From the Metabolism Division, Department of Medicine, Washington University School of Medicine and Barnes Hospital, St. Louis, Missouri.

TABLE 1

The treatment of severe diabetic acidosis: phase of extracellular fluid restoration

I. Insulin
   a. Initial: 200 to 300 units of crystalline insulin of which one-quarter to one-half may be given intravenously.
   b. Sustaining: 50 to 100 units of crystalline insulin every hour until significant fall in hyperglycemia or ketonemia has occurred. Thereafter, insulin administered less frequently and in smaller doses.

II. Fluids
   a. Initial: 1/6-M Sodium Lactate, one to two liters administered rapidly with careful clinical observation.
   b. Sustaining: Lactated Ringer's Solution. During the first four hours the total intravenous intake should be about four liters for an adult.

III. Supportive Treatment
   a. Gastric aspiration only if vomiting continues.
   b. Antibiotic treatment of any infection.
   c. Vasopressor drug treatment of hypotension not responding to fluid therapy.

An understanding of the magnitudes of the fluid and electrolyte losses in diabetic acidosis is the foundation of rational replacement therapy. Table 2 summarizes metabolic data obtained by several investigators. Considerable variation in estimate is evident but certain facts need emphasis. Firstly: Water is lost from the intracellular as well as the extracellular compartments of the body. This is indicated by a loss of water that greatly exceeds the amount of water that would be expected by simple extracellular fluid depletion as judged by the sodium losses. Secondly: Potassium deficits equal or exceed the loss of sodium and are greater than can be accounted for on the basis of the negative nitrogen balance. Thirdly: The ratio of the urinary loss of chlor-

TABLE 2

Estimated deficits in diabetic acidosis per kilogram of body weight (from Danowski)[3]

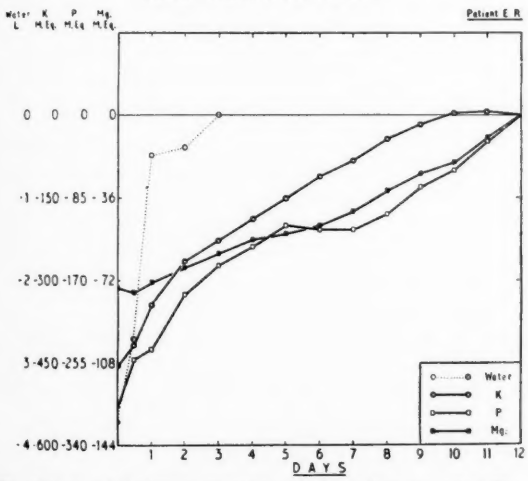
Water	87	to	114 ml.
Sodium	5.1	to	13.3 mEq.
Potassium	4.6	to	6.1 mEq.
Chloride	2.5	to	9.5 mEq.
Nitrogen	0.09	to	0.9 gm.

ide to sodium is equivalent to their relative serum concentrations in most cases.

Therapeutic zeal to restore the missing body constituents must not exceed the ability of the depleted acidotic patient to utilize administered water and salts to reconstitute the extracellular and intracellular fluids. With present therapy the volume of the extracellular fluid is rapidly restored. Sometimes the volume of the extracellular space may be expanded excessively after twelve to twenty-four hours of treatment when replacement fluids are composed largely of isotonic sodium chloride. The restoration of intracellular constituents has been shown by Nabarro, Spencer and Stowers[7] to require a longer time than the replacement of the extracellular constituents. Figure 1 is taken from their studies and shows the disparity between the rates of water and electrolyte uptake by cells. Intracellular water was restored in this case within twenty-four to forty-eight hours but the incorporation of potassium and phosphate continued over ten to twelve days. Nitrogen equilibruim was even more delayed.

The first objective of fluid therapy is the rapid expansion of the extracellular fluid compartment which has been depleted during the period of developing keto-

CELLULAR RESTORATION DURING RECOVERY FROM DIABETIC ACIDOSIS



Cumulative cell balances of water and electrolytes corrected for changes in cell nitrogen. Ordinate scales in proportion to normal concentrations of potassium, magnesium and labile phosphorus in cell water.

FIG. 1. The time required for intracellular restoration of a patient with diabetic acidosis has been charted by Nabarro, Spencer and Stowers.[4] The early restoration of intracellular water but delayed incorporation of potassium, phosphorus and magnesium are clearly shown.

acidosis. I am impressed that severely acidotic patients are benefited promptly by the administration of 1/6-molar sodium lactate. Respiratory effort decreases promptly and some of the damaging effects of acidosis per se are relieved. Lactate treatment should be guided by the clinical response of the patient; improvement in respiratory effort is usually noted with one or at most two liters of 1/6-molar sodium lactate. When the desired therapeutic effect on respiration is obtained, extracellular fluid volume replacement is continued with Ringer's solution containing lactate. This solution is definitely preferable to 0.9 per cent sodium chloride because it contains chloride at a concentration that approximates the normal concentration of chloride in the extracellular fluid. Isotonic saline, on the other hand, contains an excessive concentration of chloride; when it is given in large amounts, metabolic acidosis is aggravated by hyperchloremia.[5]

During the second phase, treatment has different objectives. Circulatory hypovolemia and severe acidosis having been overcome, attention may now be directed to providing water, electrolytes and carbohydrate for cellular reconstitution. Special electrolyte mixtures have been proposed by Butler,[6] by Nabarro and associates[7] and by others[8] for this purpose. It is convenient to utilize readily available parenteral fluids, however, in a general medical ward.

The replacement solution recommended in table 3 is obtained by giving equal amounts of lactated Ringer's solution and 5 per cent sugar. The latter solution provides free water for intracellular rehydration and continuing urinary loss. The contained glucose or fructose is helpful in promoting carbohydrate utilization and storage. During this phase of treatment the rate of fluid administration is slowed.

The addition of potassium to the parenteral fluids may now be considered. If the urinary output is good

TABLE 3

Treatment of severe diabetic acidosis (continued): phase of intracellular reconstitution

I. Insulin: Careful adjustment of dosage to provide progressive reduction of hyperglycemia without hypoglycemia. Interval between insulin injections should be prolonged to two to four hours with abating glycosuria.

II. Fluids: Equal quantities of lactated Ringer's solution and 5 per cent fructose (or glucose) are infused at a rate of 300 to 400 ml. of the combined fluids per hour.

III. Potassium: Buffered potassium phosphate, 40 mEq., added per liter of fluid with earliest EKG or laboratory evidence of subnormal potassium levels.

IV. Oral Intake: Fluids and liquid nourishment should be delayed until nausea has completely disappeared and normal gastric function is assured.

and the response of insulin has been satisfactory it is unnecessary to await clinical and electrocardiographic signs of potassium deficiency before including potassium in the administered fluids. A determination of the plasma potassium, however, is helpful at this stage in treatment in patients responding slowly to treatment. Potassium *phosphate* has an advantage over the usually employed chloride salt, because it furnishes phosphate which has been lost from the intracellular fluid rather than chloride which is already adequately provided. Forty milliequivalents of the buffered potassium phosphate may be safely added per liter of fluid. In exceptional cases higher concentrations of potassium may be required in profoundly hypokalemic patients.

In the proposed scheme intravenous carbohydrate is given to the patient after the first three to four hours. Ten years ago the use of glucose was a topic of violent controversy. Now most students of diabetic acidosis advocate the use of sugar after the blood sugar concentration has fallen significantly. The beneficial effect of carbohydrate on ketonemia has been tested under controlled conditions in man by our group.[9] Diabetic patients were allowed to enter moderate acidosis under careful hospital supervision. Regular insulin was then given in doses adequate to control hyperglycemia during the administration of 0.9 per cent sodium chloride over a four-hour period. At a later time acidosis was reinduced in the patient and either glucose or fructose was administered to the same patient receiving the same dose of insulin as was used previously. In all, twenty episodes of acidosis were studied in eight diabetic patients; therapies were directly compared in individual patients. The rate of fall of the blood ketones was measured and was found to be significantly faster in the presence of either glucose or fructose than with saline solution (figure 2). It was impossible to detect a significant advantage of fructose over glucose in respect to recovery from ketonemia but with fructose the fall in blood sugar was more prompt. It was concluded that despite initial hyperglycemia the addition of carbohydrate to the therapeutic regimen sped recovery from ketosis.

Delay in carbohydrate therapy is advisable in practice to avoid excessive extracellular fluid osmolality and profound osmotic diuresis. Glycosuria in diabetic acidosis is associated with increased losses of water, sodium and potassium.[4]

At a practical level the differences between glucose and fructose in the treatment of diabetic acidosis are small. Fructose has the advantage of being more readily utilized by the liver in diabetes but much of the fructose is transformed by the liver into glucose and re-enters

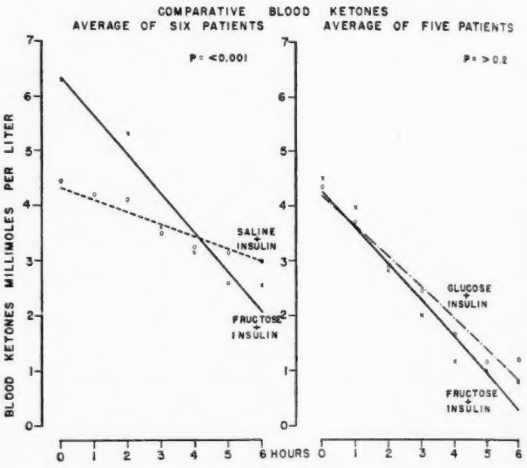


FIG. 2. A comparison of the rate of decrease of blood ketones of patients with induced diabetic ketosis. In A, the response with saline is compared to that obtained with fructose. In B the falls in blood ketones with glucose and fructose treatment are shown to be similar. (Taken from Rosecan and Daughaday.)[9]

the circulation. As previously mentioned fructose causes less elevation of total blood sugar than glucose. One possible disadvantage of fructose in the later stages of treatment is the fact that it is poorly utilized by the central nervous system. It is possible to have hypoglycemia at the same time that reducing sugar is present in the urine due to continued fructosuria. Urinalyses with enzymatic methods (glucose oxidase papers) provide more reliable guides for treatment at this stage.

Fortunately the response to treatment is uneventful and recovery occurs promptly in the vast majority of patients with diabetic acidosis. A small fraction of the cases present real therapeutic problems such as major complicating infection or other disease states. A few will develop such iatrogenic and preventable complications as hypoglycemia and hypokalemia. Although I recognize the magnitude of these problems, I would like to focus attention on the circulatory complications of diabetic acidosis. Hypotension occurs in many patients who enter the hospital in profound acidosis. Usually the blood pressure rises promptly after the administration of salt solutions. The administration of 6 per cent dextran has been advocated in the presence of hypotension.[3] The hypotension occurring in these patients can be attributed to hypovolemia. Occasionally the blood pressure does not respond to hydration and in others hypotension may first appear many hours after treatment has been instituted, and lead to death. Case reports of such unexpected

deaths have emphasized that they took place after biochemical improvement.

The mechanism of this type of shock has been clarified by the studies of Howarth and her associates.[10] Cardiac catheterization was carried out in five patients who had refractory hypotension complicating diabetic acidosis and who later succumbed. Right auricular pressures and cardiac outputs were both found to be normal or slightly elevated whereas in simple hypovolemic shock these parameters are both depressed. In view of the normal cardiac output, hypotension can only be attributed to decreased peripheral resistance which was estimated to be only half of normal. Normal protective vasoconstriction in the face of hypotension was therefore absent.

Few measurements of the circulation in different parts of the vascular bed in diabetic acidosis have been reported. Reduced blood flow in the hand, as measured by the plethysmograph, was found in acidotic patients with unresponsive hypotension by Schecter, Wiesel and Cohn.[11] Kety and co-workers,[12] in their classic studies of the mechanism of disturbed central nervous system function in diabetic coma, found increased cerebral blood flow in comatose patients with, however, decreased oxygen uptake. The increase in cerebral blood flow was attributed solely to acidosis. Renal blood flow and glomerular filtration are both decreased in diabetic acidosis. When renal hemodynamics are depressed for sufficiently long, tubular damage occurs.[13]

The factors which provoke late vasomotor collapse are poorly understood. Unrecognized hypokalemia may be a major factor but is absent in some cases. A critical review of the charts of patients with vasomotor collapse at Barnes Hospital suggests that the clinical response to treatment by these patients was usually delayed and characterized by uncontrolled hyperglycemia. Two cases treated on a general medical service have been selected to illustrate the problem of late vasomotor collapse.

The first patient (figure 3), a forty-six-year-old obese colored woman, entered the hospital in a semicomatose condition. Her acidosis was not profound as indicated by a plasma carbon dioxide combining power of 12 mEq. per liter. Her response to 500 units of insulin administered during the first four hours of treatment was not satisfactory in that the blood sugar actually rose from 800 mg. per 100 ml. on entry to 920 mg. per 100 ml. Some of this rise may be attributed to the injudicious use of glucose which contributed to the hyperglycemia. Despite the fact that the skin continued to be warm, the radial pulse strong and polyuria persistent, there was a progressive fall in blood pressure, eventually leading to

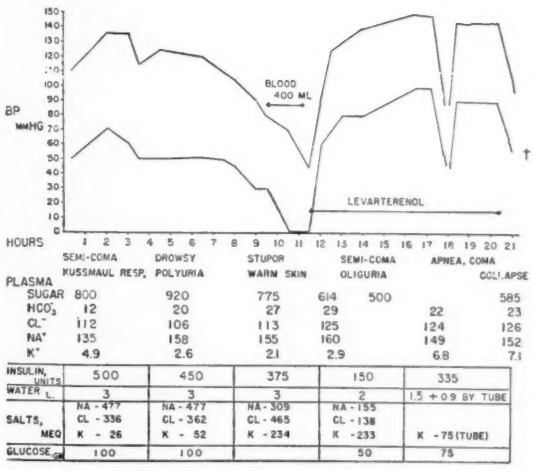
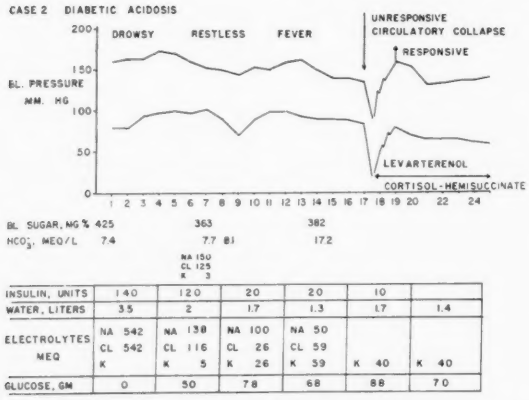


FIG. 3. The hospital course in Case 1 is summarized graphically. The patient was a forty-six-year-old obese woman who had noted the onset of diabetic symptoms three weeks previously. Vomiting and severe weakness had occurred during the day before entry. Deep breathing and lethargy supervened. Hypertension had been found in the past.

complete vasomotor collapse. Blood transfusion was without benefit but levarterenol, when finally used, did restore blood pressure to normal levels. Cerebral function, however, remained poor with weak, shallow respirations. Cardiac arrhythmias developed and eventually led to death. Hypokalemia, which developed early, may have contributed to the collapse. Later moderate hyperkalemia occurred despite the fact that careful monitoring with the electrocardiogram failed to show the expected changes of hyperkalemia.

The second case illustrates the fact that vasomotor collapse may occur late in the course of treatment. The patient (figure 4), a sixteen-year-old girl, entered the hospital in severe diabetic acidosis with a plasma bicarbonate combining power of 7.4 mEq. per liter. As she was only drowsy her condition was not considered alarming and she received only physiologic saline solution and modest doses of insulin during the initial phase of treatment. She did not respond adequately to treatment in that the plasma bicarbonate combining power had risen only to 7.7 mEq. after eight hours of treatment. Fever, without ascribable cause, restlessness and mental obtundity developed, suggesting disturbed hypothalamic function. Vasomotor collapse occurred precipitously seventeen hours after entry and was immediately recognized by the attending physicians. The benefit achieved with levarterenol was unequivocal but eventual recovery occurred slowly with gradual subsidence of the



INSULIN, UNITS	140	120	20	20	10	
WATER, LITERS	3.5	2	1.7	1.3	1.7	1.4
ELECTROLYTES MEQ	NA 542 CL 542 K	NA 130 CL 116 K 5	NA 100 CL 26 K 26	NA 50 CL 59 K 59	K 40	K 40
GLUCOSE, GM	0	50	78	68	88	70

FIG. 4. The hospital course in Case 2 is summarized graphically. The patient was a sixteen-year-old girl with acute onset of diabetic symptoms one month prior to hospitalization. Weakness had progressed during the week before entry. For one day deep breathing and progressive mental obtundity were noted. She entered the hospital comatose.

central fever and the re-establishment of vasomotor control.

Several aspects of this complication deserve emphasis:

1. Peripheral vasomotor collapse may be present on entry or develop during the course of treatment of diabetic acidosis.

2. Cutaneous signs of vasoconstriction may not occur or appear late and delay clinical recognition of the circulatory disorder.

3. Blood pressure determinations, carefully obtained at frequent intervals and faithfully recorded, permit early recognition of vasomotor instability.

4. Blood transfusions are of little benefit if hypovolemia has been corrected and may precipitate pulmonary edema. Potent vasoconstrictor drugs, such as levarterenol, when used promptly, are of real value.

In closing, I would like to emphasize the importance of careful attention to clinical details in the management of diabetic ketoacidosis. Survival of the patient is often dependent on some apparently trivial aspect of therapy. There is no substitute for the continuous attention of a competent physician. Frequent evaluation and recording of the mental condition, pulse, blood pressure, urine volume, urine sugar and acetone, blood sugar and serum bicarbonate are essential to the patient's welfare. Small details, such as the recognition of gastric dilatation or the subcutaneous infiltration of fluids, may make the difference between death and survival. There can be no doubt that painstaking clinical observation has improved the results in the treatment of diabetic acidosis as Harwood has recently pointed out.[34]

SUMMARY

The therapy of diabetic ketoacidosis has been discussed with emphasis on the importance of adequate insulin dosage and the physiologic restoration of extracellular and intracellular fluid components.

Fluid therapy has been divided into two phases. The first is the phase of extracellular fluid restitution, during which 1/6-molar sodium lactate and lactated Ringer's solution are employed. During the subsequent phase of intracellular fluid restoration and maintenance, carbohydrate-containing solutions, hypotonic in respect to electrolytes, are used.

Attention is drawn to peripheral vasomotor collapse, which may occur at any time during treatment, as a significant cause of death in diabetic coma. Early recognition and prompt treatment with levarterenol may be lifesaving.

SUMMARIO IN INTERLINGUA

Natura E Correction De Cetoacidosis Diabetic

Le therapia de cetoacidosis diabetic es discutite con referentias special al importantia de adequate dosages de insulina e del restauration physiologic del componentes del fluidos extra- e intracellular.

Le therapia a fluido es dividite in duo phases. Le prime es le phase del restauration del fluido extracellular. In iste phase, lactato de natrium de 1/6 mol e lactate solution de Ringer es empleate. In le secunde phase, que es le phase del restauration e mantenentia del fluido intracellular, solutiones que contine hydratos de carbon e que es hypotonic con respecto al electrolytos es empleate.

Es signalate le phenomeno del collapso vasomotori peripheric. Isto pote occurrer a non importa qual tempore in le curso del tractamento. Illo es un causa significative de morte in coma diabetic. Le immediate recognition e un prompte tractamento con levarterenol pote salvar le vita del patiente.

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DISCUSSION

KERMIT L. PINES, M.D.: Dr. Daughaday's excellent paper mentions the shrinking areas of disagreement in a field where tempers ran high a few short years ago. This is another example of the unfavorable influence of facts upon controversy. There is, thus, little for me to discuss, a fortunate thing at this late hour.

On the basis of a review of the experience at Presbyterian Hospital from 1940 to 1950 by Dr. Morton Binder and myself, our method for the treatment of severe diabetic acidosis was revised with apparently good effect. Perhaps the most important factor was the dramatization of diabetic acidosis to the house staff as an emergency requiring immediate and constant attention. The administration of potassium was of great value. It is worth emphasizing again that potassium may be toxic if given too rapidly or in the presence of damaged kidneys.

We agree with the speaker that it is best to err on the high side of insulin dosage, but allow the patient's early course to help us decide how rapid the increase should be. We prefer hourly rather than two-hourly insulin doses and watch carefully for a hypoglycemia later on in the course, particularly when large doses have been required.

I am glad we agree on the use of alkali. We happen to prefer intravenous sodium bicarbonate but have no objection whatever to lactate. We have never had the problem of alkalosis as a result. We have, however, seen a patient, transferred from another hospital where alkalosis was induced by administration of a liter of 5 per cent sodium bicarbonate. This patient survived.

A number of centers use premixed repair solutions. We feel that if the various aspects of diabetic acidosis are treated as suggested by Dr. Daughaday, more elasticity is permitted by the use of alkali saline, dextrose in water, and potassium chloride as indicated by the changing condition of the patient from hour to hour. The serum phosphate certainly falls, but we have not been convinced of any advantage in the replacement of phosphate; nor have we felt the need for fructose, since neither glucose nor insulin is in short supply.

TABLE 1

Severe diabetic acidosis* (1940-1957)
Medical Service, Presbyterian Hospital

	1940-1951	1952-1957
Admissions	100	60
Deaths	13	1
Mortality	13%	1.7%

* Serum bicarbonate 10.6 mEq./L. (25 vol. %) or less on admission.

Table 1 summarizes our recent experience. I might say that we do not believe that the results of today, in comparison with the results of previous years, necessarily prove the value of the method used in treatment. There are a great many other factors, I am sure, that are operative. This series, also, is very small, but it does seem to indicate that recent advances may have had some influence.

Obesity, Fat Metabolism and Diabetes

David Adlersberg, M.D., New York City*

The frequent association of obesity and diabetes has been known for many years. It led to the view that this combination represents a special form of diabetes ("lipogenic diabetes") caused by impaired glycogen storage in the liver.[1-4] According to this concept, obesity is the primary abnormality and hyperglycemia and glycosuria are secondary phenomena. This concept is based chiefly on the observation that adequate weight reduction restores carbohydrate tolerance to normal.[1,2] This concept is not shared by many.[5,6] Persistence of abnormal glucose tolerance tests after adequate weight reduction has been often observed in originally obese diabetics and after subsidence of all clinical symptoms (figures 1a & b).[6,7] The duration of diabetes, the frequency of complications, the incidence of diabetes in parents and siblings and the proportion of heavy babies are very similar among obese and nonobese diabetics (table 1) whereas one would expect considerable differences between the two types if they were etiologically distinct conditions.[6] Thus, subsidence of symptoms of diabetes does not mean disappearance of diabetes in the originally obese diabetic.

There is no proof that obesity causes diabetes. Among 170 million Americans there are fifty to sixty million overweight persons. Of these only two million have diabetes, an incidence of 3 to 4 per cent. The incidence in the general population is approximately 2 per cent. Both abnormalities may be manifestations of the same cause or causes. Some are of the opinion that an obese person after weight loss still has the anomaly that caused the obesity and may be considered "potentially obese" despite normal body weight and normal caloric balance for the very same reason that a symptom-free diabetic remains a diabetic.[8]

OBESITY AND DIABETES—CORRELATIONS

The prevalence of obesity among diabetics today appears to be higher than at the turn of the century. Frerichs observed 15 per cent obesity among his patients with diabetes, Segen 30 per cent, Bouchard 45 per cent and von Noorden 22 per cent.[9] Von Noorden considered the possibility that many of these data are too low because of weight loss prior to the discovery of diabetes. After correction, his ratio of obese diabetics rose to 35 per cent. The early figures of Joslin and Umber were 40 per cent and 34 per cent, respectively. In contrast, at least 80 per cent of the diabetic persons living at present in the United States are or have been overweight.[10]

It would be of great interest to compare the prevalence of overweight with that of diabetes or with dia-

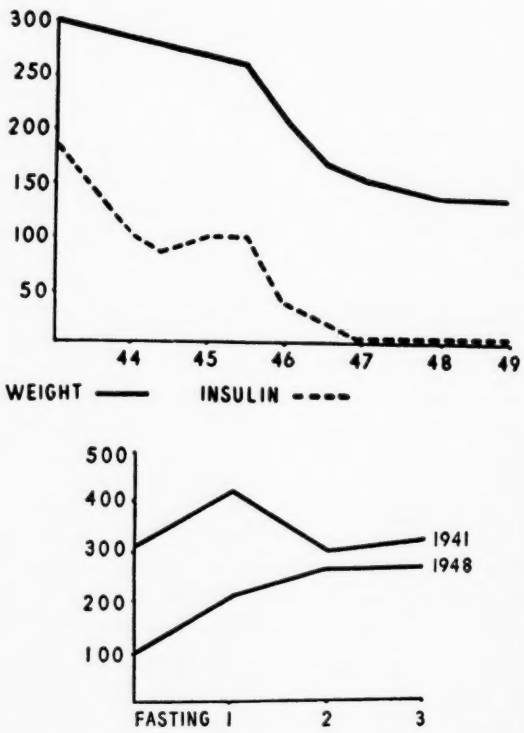

FIG. 1. (a). Marked weight reduction of an obese diabetic resulted in gradual subsidence of symptoms and signs of diabetes. The insulin dosage was reduced from 180 units in 1943 to zero in 1947.[7]
(b). Despite absence of symptoms and signs the glucose tolerance test showed a typical diabetic curve in 1948.[7]

Presented at the Symposium on Fat and Diabetes sponsored by The Clinical Society of the New York Diabetes Association, Inc., on Oct. 12, 1957.

From the Departments of Medicine and Chemistry, Mount Sinai Hospital.

TABLE 1

Duration of diabetes, frequency of complications (polyneuritis, retinitis and cataract) and incidence of diabetes in parents and siblings among persons with mild diabetes and obesity are compared with corresponding data in diabetics requiring insulin.[8]

DURATION OF DIABETES

Years of diabetes	Diabetes treated with insulin per cent	Mild diabetes with obesity per cent
0-5	64	75
6-10	20	15
11-15	13	8
16-20	2	2
over 20	1	0

COMPLICATIONS OF DIABETES

Type of diabetes	No. of patients	Polyneuritis No.	Polyneuritis Per cent	Retinitis No.	Retinitis Per cent	Cataract No.	Cataract Per cent
Diabetes treated with insulin	277	72	26	21	8	26	9.5
Mild diabetes with obesity	107	21	19.6	8	7.5	6	5.6

HEREDITY

Type of diabetes	No. of patients	Diabetes in parent or sibling
Diabetes requiring insulin treatment	277	32
Mild diabetes with obesity	107	24

Table 1 is based on data presented by G. O. Richardson, M.D., Newcastle upon Tyne, England, in an article entitled "The Obese Diabetic," which appeared in DIABETES 2:454-56.

TABLE 2

Death rates from diabetes in nine countries[10]

Country	Diabetes mortality* 1948	1938	1928
United States	26.4	23.9	23.6
Canada	20.3	13.8	11.1
Finland	5.9	8.1	—
Sweden	6.8	10.8	12.6
France	7.3	10.1†	—
Switzerland	11.2	16.2	10.3
England—Wales	7.6	11.5	13.1
Australia	18.8	17.7	12.0
New Zealand	20.1	18.8	12.2

*Rates per 100,000 population
†1934 data

betes mortality in various countries since such a comparison might perhaps explain the geographic variations in diabetes mortality (table 2).[10] Unfortunately, there are no collected data on overweight. There is some indication that after correction for difference in height, American men are about 10 lb. heavier and women 4 to 6 lb. heavier than their English counterparts.[11] Hundley[10] attempted to correlate the percentage gain in average body weight between ages twenty-five to sixty with diabetes mortality rates after age forty-five in four popu-

TABLE 3

Increase in average body weight between twenty-five and sixty years versus diabetes mortality[10]

Country*	Weight increase between ages 25 and 60 years Men %	Women %	Diabetes mortality† Over 45 years of age Men	Women	All ages Men	Women
Japan	0.4	0.4	?	?	2.4	2.0
England	3.6	15.5	14.0	25.2	5.3	10.1
Canada	4.6	19.3	55.6	91.5	16.2	24.5
United States	8.1	15.0	67.7	111.6	20.7	34.0

*Japanese men, 63.7 in. in height—women, 59.5 in. (their national average in 1949); English and Canadian men, 66 in. in height—women, 62 in. (without shoes); U.S. men, 67 in. tall—women, 63 in. (with shoes).
†Mortality rates per 100,000 population in 1948.

lations in the year 1948 (table 3). In three populations with marked weight increases higher diabetes mortality rates were observed (England, Canada, United States of America, in comparison with Japan). Nevertheless, diabetes mortality varied considerably among the first three countries probably due to some additional factors. To explain the high mortality rates from diabetes in the United States, three possible factors have been considered: generous diet consumed by many in excess, increasing use of labor-saving devices and the prevalence of overweight in adults. It is of interest that a downward trend in diabetes mortality in the United States has been observed since the late 1940's. Diabetes mortality in 1954 was 9 per cent less than in 1949 despite the increasing proportion of old people in the population.[12] The possibility that this decrease in mortality may be due, at least in part, to the growing awareness of the importance of weight control must be considered.[10]

Many objections can be raised against the correlations mentioned above. Such factors as improved diagnosis of diabetes, regional variations in statistical procedures, prolongation of life in general, and especially in persons with diabetes, by better treatment, and perhaps differences in many environmental influences (socio-economic, occupational, nutritional, climatic) may explain many of these observations. Comparative epidemiologic studies in diabetes as well as in other diseases (atherosclerosis) are exposed to this criticism.

CARBOHYDRATE TOLERANCE IN OBESE PERSONS WITHOUT DIABETES

The impaired carbohydrate tolerance appears to be related chiefly to the duration of obesity. In the Nutrition Clinic of The Mount Sinai Hospital, Drs. Weil and Williams studied the glucose tolerance in fifty-four obese persons, predominantly women (unpublished observations). Only persons who had no glycosuria and normal

fasting blood sugars were included. They were divided into two groups. Nineteen persons had obesity for several years only; they were in the "dynamic" ("active") phase of weight gaining during the study. The other group of thirty-five were in the "static" phase with a history of steady overweight for ten to twenty-five years. The average age of the "dynamic" group was 33.5 years, that of the "static" 46.4. The average ideal weight was 127 lb. in the "dynamic" group and 129 in the "static." The average actual weight was 188 and 205 lb., respectively, and the average overweight expressed in per cent of ideal weight was 27 and 57 per cent, respectively. Thus, the "static" group consisted of older, heavier and longer-obese persons than the "dynamic" group. Persons of corresponding ages and normal body weight served as controls. The data are summarized in figure 2.

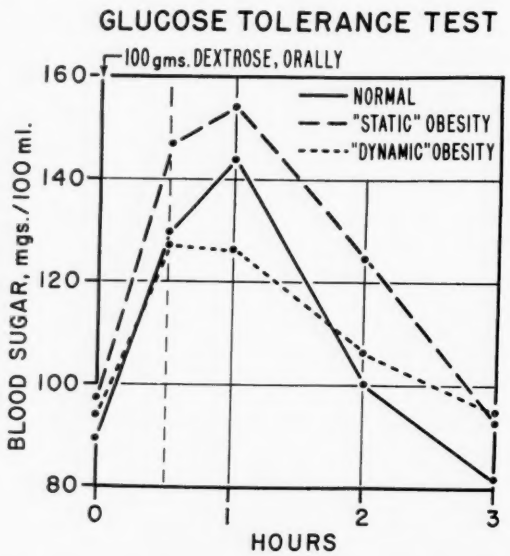

FIG. 2. Glucose tolerance tests in "static" and "dynamic" obesity and in normal controls. Note lower average curve in "dynamic" obesity. For details, see text.

A trend without statistical significance is indicated in that persons in the "static" phase of obesity exhibit higher blood sugar curves than those in the "dynamic" phase in agreement with the observations of Beaudoin et al.[13] These findings might be explained by differences in actual weight, age and duration of overweight between the two groups. Age may be a factor per se in that the probability of decreased carbohydrate tolerance increases with age.[14] The assumption of basic differences between the two groups, such as decreased oxidation of fat and proportionately increased oxidation of glucose is speculative and unproved.

GENETIC ASPECTS

There are only limited observations on the hereditary aspects of the common variety of obesity. Separation of genetic from environmental factors in the etiology of obesity is difficult. Among parents of obese patients, the incidence of overweight and of diabetes was two to ten times normal.[15] When both parents were obese 73 per cent of the offspring showed this abnormality; if only one parent was obese, 41 per cent of the children were found to be obese; if both parents had normal weight, only 9 per cent of the children were obese.[16] That body build is inherited is well known. Hereditary factors seem to play a role in the genesis of obesity, the importance of which is difficult to evaluate. The dictum of Newburgh,[17] "Body build is inherited; obesity is not," is perhaps too dogmatic. A modified version is suggested: "Body build is inherited, obesity *perhaps* inherited."

Family studies in obese diabetics reveal 25 per cent incidence of diabetes and 60 per cent incidence of obesity.[4] The twin study method has been applied extensively to investigations concerning the contribution of heredity to pathological states. A pair of monozygotic twins studied by Warkany et al.[18] is of interest in this respect. The two brothers were concordant in all blood factors studied. They became discordant in the eighth year of life when one twin became diabetic and remained lean and small despite adequate treatment whereas the second twin became obese. He remained free from diabetes up to the end of the follow-up period at age twenty-eight. It is well known that concordance in diabetes is much more frequent in monozygotic than in dizygotic twins. In the above-mentioned family diabetes occurred in the mother's family and obesity in the families of both parents. The observation by Warkany et al. again suggests a close etiologic relationship between obesity and diabetes mellitus. One might assume that a single gene or perhaps two closely-related genes might be responsible for the two metabolic abnormalities.

Among nonglycosuric obese as well as nonobese family members of obese adult diabetics, in our experience, abnormal glucose tolerance tests are not infrequently seen. The frequent combination of obesity and diabetes in adults supports the concept that human obesity may represent a hereditary trait accompanying diabetes. This would be in accord with Mayer's observations in obese-hyperglycemic mice. To paraphrase Joslin, diabetes is a punishment of obesity but probably only in the predisposed person.

The treatment of the obese diabetic must be primarily directed against obesity. It is well established that weight reduction achieves in many instances full control of symptoms of diabetes. The easy way of treating this form of diabetes with insulin or tolbutamide is inadvisable and should be used only as an exception to the rule. Some consider insulin "contraindicated" in the obese diabetic except for complications.[5]

SERUM LIPIDS IN DIABETES

The relationships between lipid and carbohydrate metabolism have been extensively studied. Very important observations have been presented in the papers of Doctors Stadie, Dole, Albrink and Man, Olson and others earlier in this Symposium. The possible implications of these studies for the pathogenesis and perhaps therapy of diabetes mellitus, obesity and atherosclerosis remain to be investigated.

Our own studies were concerned with the serum lipid partition in diabetes. Extremely high serum lipid levels have been observed occasionally in patients with diabetes mellitus.[19] The highest reported figure of total lipids is 48 gm./100 ml.[20] However, this figure obtained by the centrifuge method for butter fat is open to criticism. Other high figures for serum total lipids range from 15 to 20 gm./100 ml.[21] The highest value of our observation was 16 gm./100 ml. It was seen in absence of ketonuria in one of our five patients with both diabetes mellitus and idiopathic hyperlipemia.[22] Since then, we have observed three additional cases of this rather rare syndrome.

These patients present alterations of lipid metabolism characteristic of idiopathic hyperlipemia often in association with skin xanthoma. The appearance of the serum is milky or creamy in the fasting state. There is marked elevation of serum triglycerides and of total lipids with a moderate to marked increase of serum cholesterol and phospholipid. The average level of total lipids in this group was 8,282 mg./100 ml. and the highest cholesterol level was 1,480 mg./100 ml. The diabetes encountered in this group is usually mild; only two patients among the eight required small quantities of insulin. In the other cases, good control of the diabetes could be achieved on lowered intake of calories and fat.

Patients with this syndrome reveal a remarkable lability of serum lipids in connection with the hyperglycemia and glycosuria even in absence of ketonuria. To illustrate this, the above-mentioned patient presented on admission marked turbidity of the serum, serum total lipids of 16,000 mg./100 ml. and total cholesterol of 660 mg./100 ml. The fasting blood sugar was 300 mg./100 ml. There were eruptive xanthoma of the skin and lipemia retinalis. A low calorie regimen without insulin resulted in a loss of 12 lb. in weight (from 187 to 175 lb.). The turbidity of the serum was reduced to 1+, total lipids declined to 2,500 mg./100 ml. and serum cholesterol to 329. This observation was made in 1948. A similar sequence of events was observed five years later in connection with overeating. The weight of the patient increased, hyperglycemia and glycosuria reappeared with recurrence of eruptive xanthoma, marked hyperlipemia and hypercholesteremia. A low calorie diet again resulted in loss of weight, disappearance of glycosuria and decrease in blood sugar, serum total lipids and serum cholesterol (table 4). The patient presented similar episodes of hyperglycemia and enhanced hyperlipemia and hypercholesteremia in 1954 and again in 1956.

Milder instances of a similar nature may have been observed by Hirsch et al.[23] and Appel and Hansen[24] whose patients exhibited marked elevation of esterified fatty acids of the blood in the presence of hyperglycemia. In this connection, recent observations by Dole and Gordon on the relationship between nonesterified fatty acids of the plasma (NEFA), lipid transport and glucose metabolism deserve emphasis.[25,26]

Complications of human diabetes may be associated with well-defined changes in serum lipids and serum polysaccharides in the absence of ketosis or acidosis.[27] Patients with severe retinopathy, hypertension, edema and proteinuria (Kimmelstiel-Wilson syndrome) show decided elevation of all serum lipid fractions and of complex carbohydrates. It is of interest that a group of diabetics with early retinopathy but without any evidence of renal involvement, present significant differences in comparison with patients having uncomplicated diabetes and with nondiabetics, consisting of increase in serum triglycerides and total lipids, in serum glucosamine and total serum polysaccharides while serum cholesterol and phospholipid remain normal (table 5). One must consider, therefore, that those blood changes perhaps precede the degenerative alteration of the tissue and the deposition of certain protein-lipid and protein-carbohydrate compounds in the retina and in the renal glomerulus.

LOW FAT DIET IN DIABETES

The possible relation between diet, and especially dietary fat, and atherosclerosis has been the topic of many studies. Regardless of all arguments, a nutritional regimen preventing obesity, which certainly is a health hazard in general and especially in the manifest or potential diabetic, appears to be justified. Such a diet should be nutritionally adequate and should provide only 25

TABLE 4

Summary of data of a patient with mild diabetes mellitus and idiopathic hyperlipemia during three observation periods in 1948, 1953 and 1954[22]

Date	Blood sugar mg./100 ml.	Urine sugar per cent	Serum turbidity	Serum Total lipids mg./100 ml.	Serum Neutral fats mg./100 ml.	Serum Cholest. Tot. est. mg./100 ml.	Serum Phospho-lipids mg./100 ml.	Body weight lbs.	Remarks
1948									
Admission	300	4+	+++	16,000		666		187	Eruptive xanthoma Lipemia retinalis
After 4 weeks	167	Tr.	+	2,500		329		175	Low calorie diet
1953									
Admission	248	4+	+++	6,700		790			Eruptive xanthoma Lipemia retinalis (?)
After 25 days	148	0	±	1,400		393			Low calorie diet
1954									
4/16	310	4.5	+++	4,470	3,231	594/385	645	175	Free diet
4/23	247	2.8	+++	2,890	2,020	382/244	488	174½	1,200-calorie diet
5/7	200	1.0	+	1,140	482	320/244	338	169¾	1,200-calorie diet
5/21	148	0.3	±	940		496/270		169	1,200-calorie diet

Serum cholesterol was determined by the Sperry-Schoenheimer method, phospholipid by Sperry's modification of the Fiske-Subbarow method and total lipids by the Bloor method. Serum neutral fat was calculated by subtracting the figures for total cholesterol plus phospholipid from that of the total lipids.

TABLE 5

Serum constituents in nondiabetic controls and in diabetics with and without complications[27]

Group	I Normal controls (N)	II Uncomplicated diabetes (D)	III Diabetes & retinopathy (D_R)	IV Diabetes & Kimmelstiel-Wilson syndrome (D_{KW})
Cholesterol, mg. %				
Total	245±54	236±49	256±41	318±64
Esterified	——	174±37	187±22	221±59
Phospholipids, mg. %	214±38	259±47	290±33	326±61
Total lipids, mg. %	629±136	804±182	958±160	1,195±268
Neutral fats,* mg. %	231	310	411	550
Glucosamine, mg. %	97±8	106±16	136±18	168±30
Polysaccharide, mg. %	146±11	139±11	158±20	181±23
	Probability values (p)			
	D vs. D_R.	D vs. D_{KW}	D_R vs. D_{KW}	
Cholesterol, total	0.23	0.0001	0.02	
Phospholipids	0.035	0.0001	Not significant by inspection	
Total lipids	0.018	0.0001	0.023	
Glucosamine	0.0001	0.0001	0.0025	
Polysaccharide	0.00015	0.0001	0.013	

*Those are calculated figures; no standard deviation is given.

per cent of the calories from fat instead of the customary 45 per cent. Thus, a 2,000-calorie diabetic diet should include only 500 calories from fat per day (approximately 60 gm.). It was shown long ago in extensive studies that excessive amounts of fat result in impaired carbohydrate tolerance in animals and man. These studies also were performed in patients with diabetes in short and long periods of isocaloric nutrition in which

the content of carbohydrate, protein and fat varied from limited to excessive amounts.[28] Low-fat diets have been recommended for patients with mild diabetes treated without insulin as well as for those with the severer variety requiring insulin. Because of the important role of obesity in diabetes and because of the possible relationship between high-calorie and high-fat diets and atherogenesis, the use of low-fat regimens in the therapy of diabetes deserves re-emphasis.

SUMMARY

Over 80 per cent of adult diabetics are or were obese. In the predisposed person, diabetes is a "punishment of obesity." Obesity is probably an hereditary trait accompanying diabetes. The treatment of the obese diabetic must be primarily directed against obesity.

Obese persons without manifest diabetes tend to exhibit after ingestion of glucose higher blood sugar curves during the "static" phase than during the "dynamic" phase. In the sample observed, the findings could be explained by differences between the two groups in actual weight, age and duration of obesity.

Extremely high serum lipid levels may be occasionally observed in patients with diabetes mellitus, even in absence of ketonuria. The highest values of our observation were encountered in patients presenting the association of idiopathic hyperlipemia and diabetes mellitus. A remarkable lability of serum lipids in connection with hyperglycemia and glycosuria, even in absence of ketonuria, is a characteristic feature of this syndrome.

Diabetes complicated by retinopathy, hypertension, edema and proteinuria (Kimmelstiel-Wilson syndrome) may be associated with elevation of all serum lipid fractions as well as of complex carbohydrates. In early diabetic retinopathy without evidences of renal involvement, increases in serum triglycerides and total lipids were observed while serum cholesterol and phospholipid remained normal. Simultaneous elevation of serum glucosamine and total serum polysaccharides was seen.

The use of otherwise adequate low-fat diets is suggested in order to combat an important associated disturbance of diabetes, obesity, and perhaps atherogenesis.

SUMMARIO IN INTERLINGUA

Obesitate, Metabolismo De Grassia, E Diabete

Plus que 80% del diabeticos adulte es o esseva obese. In le subjecto predisponite, diabete es un "punition pro obesitate." Il es probabile que obesitate es un tracto hereditabile que accompania diabete. In le caso del diabeticos obese, le tractamento debe occupar se primarimente del obesitate.

Subjectos obese sin diabete de forma manifeste tende a reager al ingestion de glucosa per plus alte curvas de sucro sanguinee durante le phase "static" que durante le phase "dynamic." In le exemplos studiate, le datos esseva explicabile per differentias inter le duo gruppos quanto al peso actual, al etate, e al duration del obesitate.

Altissime nivellos de lipido seral se observa a vices in patientes con diabete mellite, mesmo in le absentia de cetonuria. Le plus alte valores in nostre experientia esseva incontrate in patientes in qui hyperlipemia idiopathic esseva associate con diabete mellite. Un aspecto characteristic de iste syndrome es le remarcabile labilitate del lipidos seral in connexion con hyperglycemia e glycosuria, mesmo in le absentia de cetonuria.

Diabete complicate per retinopathia, hypertension, edema, e proteinuria (syndrome de Kimmelstiel-Wilson) pote esser associate con un elevation de omne le fractiones de lipido seral como etiam de carbohydratos complexe. In casos de retinopathia diabetic precoce sin evidentia de un affection del renes, augmentos del nivellos seral de triglyceridos e de lipidos esseva observate, durante que le valores de cholesterol e de phospholipido del sero remaneva normal. Esseva observate elevationes simultanee de glucosamina e del polysaccharidos total del sero.

Es proponite le uso de dietas a basse contento de grassia sed alteremente adequate pro combatter obesitate —que es un importante disordine associate con diabete —e forsan etiam atherogenese.

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DISCUSSION

HOWARD ROOT, M.D.: Dr. Adlersberg has given us food for thought in this very interesting paper, which evaluates the importance of several factors related to human diabetes and places special emphasis on lipid metabolism and obesity. He has pointed out basic clinical factors that contribute to mortality rates.

I should like to stress again the fact of the increasing duration of life of diabetic patients. The cases with onset of diabetes before fifteen years of age, who have died in recent years, have actually lived ten to twenty times as long as those children who had similar onset and lived in the period before insulin. The average duration of diabetes has doubled in middle life. That factor itself must influence vital statistics which deal with such complications as coronary arteriosclerosis, retinitis, et cetera. The actual expectation of life of those children has now reached a length of forty-four years.

I am very much interested in his emphasis on heredity. We recognize in our diabetic patients a multiple-type of heredity. It carries with it some special features that we cannot as yet define very well. The inheritance of food habits is one thing. The heredity of the body build is another. Recently we have had two patients in whom necrobiosis lipoidica diabeticorum was present for a long period of time before any clinical or chemical evidence of diabetes occurred. One was a child of ten years, the second, a woman of thirty-five years who got necrobiosis long before blood sugar tests disclosed diabetes. Other features, possibly illustrating heredity, interest us. For example, the children of diabetic mothers gain weight and become quite rotund by the time they are five, six, or seven years, even though the parents have made efforts to prevent such gains in weight.

The mild diabetes of the obese middle-aged, the so-called mild diabetes that we hear discussed, nevertheless produces in that man's son or daughter, or his grandson or granddaughter, the same kind of severe brittle unstable diabetes as is found in other juveniles.

Heredity, then, is a major feature which gives a malignant character to the diabetic problem of today. The fact that one out of four people is estimated to carry the diabetic heredity factor and that he may not transmit it to his sons or daughters, but may pass it on to more distant relatives, is worth remembering.

The incidence of heredity becomes higher the longer you observe patients. I have just studied 247 of our patients who have had diabetes for over thirty-five years, and already over 60 per cent of those now know of diabetes in their families. So, I appreciate what Dr. Adlersberg has said about heredity.

I think the lipoprotein analyses in our diabetics have also emphasized the fact that elevation of serum lipoproteins occurs at the time when diabetic patients are under poor control, when they begin to turn the corner,

when severe forms of retinitis are beginning. There is something about that elevation in the lipids, particularly the lipoproteins, which marks a milestone in the development of the Kimmelstiel-Wilson disease, when those values are highest.

I would like to corroborate all that he has said about diet. I am sure that we are all going to be more and more interested in emphasizing the necessity of diet in the control of diabetes today.

HERBERT POLLACK, M.D.: Dr. Adlersberg has really presented a kaleidoscopic summary of a very vast problem. We do not know too much about diabetes, we do not know too much about atherosclerosis, we do not know too much about lipoproteins, but we surely know a lot about obesity. So we can talk a lot about obesity and its cure.

Let's take the energy balance story of the obese people, and let's give just one possible explanation as to why they might develop diabetes.

By the rule of thumb, which works out fairly well, you know that the basal caloric requirements of an individual are approximately ten calories per pound. If we take the 150-lb. normal-weight individual, we can assume that his basic requirements are in the order of 1,500 calories. Next we take a moderately obese person of 200 lb., and we can assume that the basic caloric requirements are in the order of 2,000. That is a difference of 500 calories a day that the obese person must supply in order to maintain his basal metabolic processes. We know from the R.Q. studies that approximately two fifths of this is in the form of carbohydrate —these extra calories—which means that the obese person is burning approximately 200 calories of carbohydrate, or approximately 50 gm. of carbohydrate, a day more than the nonobese. This means that he requires more insulin—about 15 units of insulin more than is required by a normal-weight individual.

If we have a pancreas and we burden it with this extra load day in and day out, then we can readily appreciate the possibility of decompensation.

One of the manifestations, as Dr. Adlersberg showed, is that when weight reduction is accomplished you frequently can restore, at least temporarily, the glucose tolerance. Because of the reduced metabolic load, the pancreas is able to handle it.

This is probably one of the explanations, or at least it is an explanation, of this phenomenon which we observe between weight loss and insulin requirements, and weight gain and precipitation of diabetes.

Just one word about diet; that is, I just want to make a plea that when we focus our attention on the diabetic and his diet, let us not forget that his nutritional requirements are still those of a normal person, and that the diabetic diet should not be perverted in any sense whatsoever. We are limited in the amount of carbohydrate that we can prescribe for a diabetic patient if we are using insulin and if our aim, in therapy, is control of glycosuria and hyperglycemia. When the carbohydrate gets much above 200 or 250 gm. a day, complete control of glycosuria is virtually impossible with the types of insulin we have today, because the postprandial plethora of carbohydrate will inevitably result in postprandial glycosuria.

With a limitation of a maximum of 250 gm. of carbohydrate, and with a total caloric intake of 1,500, and with the limitations of protein in the way of economic and satiety requirements, then, obviously, the rest of the caloric intake must be supplied by fat. We have no choice. And to state categorically that you cannot give more than 100 gm. of fat a day is a mistake.

The diet must be tailored to the individual. It must be tailored to his total caloric requirement. The limitations of the diet are such with respect to carbohydrate and protein that the difference must be supplied by fat in order to give him that amount of calories to maintain his optimum weight. In a 1,000-calorie diet one usually includes 100 gm. of protein. This amount of protein supplied by the common foods will include about 40 gm. of fat or, expressed in terms of calories, 360. Thus the low calorie diets have as high as 36 per cent of the calories from fat sources. It would be most difficult to keep the fat content to 2-5 per cent of the calories.

DR. ADLERSBERG: I wish to thank the discussers for their remarks.

I am glad that Dr. Root stressed the importance of heredity. This is really one leading factor, frequently neglected, but important for our understanding of many clinical observations in patients with diabetes.

It is interesting that in two of Dr. Root's patients, necrobiosis was the initial symptom of diabetes. The same may be seen in other complications. We have observed a number of cases in whom retinopathy of the type seen in diabetes or neuropathy was present before glycosuria and hyperglycemia became apparent.

I would like to emphasize that the classical high fat diets originated in the pre-insulin era when they were a necessity. Today the fat content of the diabetic diet may be similar to that of nondiabetics amounting to 60 to 80 gm. per day. The rest of the calories is covered by carbohydrates and somewhat larger quantities of proteins. The use of the old-fashioned high fat diets in the treatment of diabetes is not justified in 1957.

EDITORIAL

HEREDITY AND DIABETES

It is now almost twenty-five years since Pincus and White[1] presented quantitative evidence showing that susceptibility to diabetes is probably determined by a recessive gene. The great variability in the age at onset and in the clinical expression of the disease have, however, caused many investigators to be reluctant to accept a single gene hypothesis. It is argued that diabetes comprises a group of diseases, and that, therefore, several different genes must be involved in causing susceptibility to the disease, recognized clinically as diabetes. Nevertheless, it remains true that the data from all studies involving the families of large numbers of diabetics may be explained on the assumption of a recessive gene as the cause of susceptibility to diabetes.[2, 3] It is not established, however, that all cases due to a simple recessive gene are due to a change at the same genetic locus, i.e., the same gene. Neither clinical data nor genetic data have been of help in resolving this problem. Clinical data, because several types of diabetes (juvenile, adult, "brittle," stable, diabetes in thin individuals, and diabetes in obese individuals) may occur within a family; genetic data, because the method of collecting the data has been such as to preclude the obtaining of information to answer this question. We shall return to this at a later point.

We may accept as the best hypothesis to explain the available data, the hypothesis that those liable to diabetes are homozygous for a recessive gene, which we will symbolize as *d*, i.e., diabetics, and those liable to diabetes are *dd*. Estimates based on two different sets of data from this country indicate that about 5 per cent of the population of the United States are homozygous (*dd*) for the gene determining susceptibility to diabetes.[2] Because of the variability in the age at onset and in the severity of the disease, only about 1 per cent of the population is recognized to be diabetic.[4] It appears from various studies that an additional 1 per cent of the population is diabetic but not recognized to be so.[4] Hence some 60 to 90 per cent of those who are genetically liable to diabetes are not recognized by present routine methods of examination. The necessity for detection programs is obvious.

If we accept the estimate that approximately 5 per cent of the population are *dd* we may estimate the probability that given individuals are liable to diabetes as a function of their age, the age of their parents, and their relation to other affected individuals.[3] For example, if an individual has a diabetic sib and neither of his parents is diabetic, but his parents are aged, the probability that he is liable to diabetes is 25 per cent. Similarly, if one parent is diabetic the probability is 50 per cent. On the other hand, if the parents are relatively young, these probabilities become approximately 35 and 60 per cent, respectively. The probability that an individual is genetically liable to diabetes if both parents are diabetic is 100 per cent regardless of the age of the parents. Using the same assumptions, it has been computed that an individual with no diabetic sib but with an affected parent or two affected paternal or maternal grandparents has approximately 20 per cent chance of being *dd*. The risk for being liable to diabetes increases as more relatives are known to be diabetic. Knowledge of the risk of developing diabetes will influence the detail and the frequency of examinations for the disease; the greater the risk, the more vigorous the preventive measures the patient and physician are willing to undertake.

The cause or causes of the great variability in age at onset of diabetes remain obscure. It has been suggested that there is a relation between the age at onset in an affected parent and the age at onset in his affected child.[5] Analysis of several sets of data, however, has shown that this phenomenon is statistical and not biological.[6] It has also been suggested that the presence of diabetes or the prediabetic state in the mother could lead to an earlier onset of diabetes in the child.[7] There does not, however, appear to be statistical evidence to support this concept.[8] Pregnancy has been suggested as a precipitant of diabetes,[7, 9, 10, 11] but the data do not appear to be convincing.[8]

Evidence from twins[12] and from various theoretical considerations[9] strongly suggests that environmental factors as yet unidentified are of importance in determining the age at onset. As Guest and Warkany[12] have suggested, intensive longitudinal studies of identical twins, of whom at least one is diabetic, could provide important information toward a solution of this problem.

The most satisfactory data to resolve the question of how many loci are concerned in determining susceptibility to diabetes would be obtained with a reliable test for the prediabetic subject. Such a test may soon be

available,[13] but more time is needed before we can be convinced of its reliability. In the absence of such a test, an answer may be derived from (a) a series of families selected because both parents are diabetic (it is important that no attention be paid to the condition of the children at the time of selection of the families) and followed until all the children have passed age sixty or have died; (b) a series of families selected because one of the parents is diabetic (here again no attention must be paid to the condition of the children at the time of the selection of the families) and followed until all the children have passed sixty years of age or have died.

There is doubt concerning the sex ratio among those who became diabetic after the age of forty,[2, 9, 11] and concerning the relationship between parity and the frequency of onset of diabetes among women past the age of forty. While neither of these is directly a genetic question, both have bearing on the nature of the data collected for genetic studies, and both have bearing on the mode of expression of the gene(s) believed to cause susceptibility to the disease. A satisfactory answer to these questions could be obtained by following until death a group of men and a group of women of known parity, and known by examination not to have been diabetic at age forty.

The data needed to answer the several questions raised in the above paragraphs are not easily collected by any single center. A cooperative project, with several centers gathering the desired data, could supply information leading to an understanding of these problems. Such knowledge may shed light on some of the environmental factors which precipitate diabetes in those who are genetically liable to the disease. Physicians may then be in a position to prevent (or to reduce the likelihood of) the occurrence of diabetes in those genetically liable to the disease and thus be a major step closer in their conquest of diabetes mellitus.

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Leonid V. Sobolev
1876-1919

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Leonid Vasiljevich Sobolev was born in 1876 in Trubchevsk, near Orel, Russia. He attended primary school at Karachev and secondary school at Novgorod-Severski. In 1893 he entered the Imperial Medical Military Academy at St. Petersburg, with which he remained connected throughout his career. Independent research which he did on diverticulosis of the colon while still a student in the Department of Histology and Pathological Anatomy, then under the direction of Prof. K. N. Vinogradov, was awarded the Iljonskay prize. In 1898 he was graduated with honors and was appointed Adjunct Assistant in the Pathology Department. He at first com-

The author wishes to express her gratitude to the Directors of the Central Medical Library of the Department of Public Health, in Moscow, and the University Library, Rostov, who supplied her with all the necessary Russian material.

bined this with clinical work under Prof. Sirotinin, but gave it up for lack of time.

In August, 1899, he began to investigate the internal secretion of the islands of Langerhans. When he was only twenty-four Sobolev received his doctoral degree for work so outstanding that it must be considered in the same class as the brilliant investigations of von Mering and Minkowski in 1889, on which Sobolev built, and those of Banting and Best in 1921, for whom Sobolev's work was a starting point.

It is a matter of great regret that in our accelerated era the publications of such distinguished scientists are rarely read. Sobolev's most important papers were originally published in German as well as Russian, but even in Russia recognition came quite late and the full scope of his work is not yet completely grasped.* Elsewhere it is little known and rarely cited. It seems well worthwhile to examine its merits in detail here.

The experimental part of Sobolev's thesis material was worked up in the physiology laboratory directed by Prof. I. P. Pavlov; the morphological investigations were carried out in the Department of Pathology under Professor Vinogradov. Its title was "Contribution to the Morphology of the Pancreas following Ligation of its Duct, in Diabetes and in Some Other Circumstances, an Experimental and Pathological-Anatomical Investigation."

It was not at all a matter of chance that Pavlov was extremely interested in these investigations and even took an active part in the experimental work. Was it not Pavlov[1] who, while a student at the Imperial Military Academy of St. Petersburg, spent the summer of 1877 working in the laboratory of the renowned physiologist, Professor Heidenhain in Breslau, where he ligated the pancreatic duct in rabbits in order to study the morphological changes in the gland caused by the retained secretion?

As far as I can determine from the literature, Pavlov[2] was the first to use the microscope to make such examinations. He also observed atrophy of the parenchyma microscopically and was struck by the great number of lymphatic follicles in the interlobular and interacinic connective tissue. It is very probable that Pavlov interpreted as lymphatic follicles what he did not know were the islands of Langerhans. (Kühne and Lea[3] in 1882 had interpreted the islands as lymphatic structures.)

It is therefore quite understandable that Pavlov performed the first ligations of the pancreatic duct for Sobolev's experiments. In 1899, while doing routine microscopic examinations, Sobolev was fascinated by the islands of Langerhans which, according to him, could only be structures for internal secretion. He based this opinion on the absence of ducts and the very intimate relation with the tortuous capillaries. He stated the hypothesis[11] that it is the islands which, as anatomically and functionally independent structures, control carbohydrate metabolism, and tried to prove this by experimental and morphological research. It was known to Sobolev that von Mering and Minkowski[4,5] (1889 and 1890) had proved experimentally that total pancreatectomy in dogs resulted in a fatal diabetes, and that no diabetes was found when, after ligation of the Wirsungian duct, the pancreas had atrophied to a small string of connective tissue. It was thus clear that something persisted, and that something, in Sobolev's opinion, could only be the islands. Sobolev established that after ligation of the pancreatic duct in rabbits, dogs and cats there was no glucosuria and that the islands persisted. (Schulze[6] independently arrived at the same conclusion after tying off the pancreatic duct in guinea pigs.) Sobolev successfully grafted, in two dogs, the vertical part of the pancreas subcutaneously. A month after the operation, the fistulate duct of the graft closed and secretion stopped. After fifty and 130 days, respectively, the transplant was removed and on microscopical examination showed severe atrophy of the parenchyma, while the islands remained intact. Once more the resistance of the islands had been demonstrated.

Sobolev also studied the functional states of the island cells. He observed more than the normal number of granules in the protoplasm of the island cells after fasting. But in animals fed preponderantly on carbohydrates and receiving glucose intravenously, only a few granules were observed. This was even more evident in a dog with two thirds of the pancreas removed; here the island cells had atrophied. Sobolev concluded that the cells produce a granular substance which disappears when the organism must utilize a lot of carbohydrate.

Sobolev had noticed in guinea pigs as well as in rabbits two distinct types of granule-containing island cells. The majority of cells did possess fine granules in their protoplasm, but in addition, and usually located peripherally, bigger cells with coarser granules were observed. Sobolev did not pursue this observation. It required pathologic-anatomic investigations to furnish him with a clinical proof of his experimental conclusion that the islands are to be considered the morphological substratum of an internal secretion which controls carbohydrate metabolism.

*In 1950 Sobolev's thesis was reprinted in Moscow with an introduction by Prof. Rossiiski, from which I have borrowed some biographical data.

Sobolev expected intact islands in sclerotic and atrophic pancreases belonging to persons who had not suffered from diabetes, and indeed they appeared to persist and proved to be more resistant than the parenchyma. In pancreases belonging to persons who had died of diabetes, Sobolev expected qualitative or quantitative changes in the islands. In thirteen out of fifteen cases, quantitative changes were found; in four cases not one island was present, and in nine cases fewer than the normal number were seen. Moreover, atrophied and vacuolated island cells were observed in two cases. Thus atrophy and hydropic degeneration of island cells were described and drawn by Sobolev at the same time as, but independently of, Weichselbaum and Stangl.[7,8] Sobolev concluded that in diabetes the islands are less resistant, sometimes disappearing via atrophy. In fact, according to Sobolev diabetes could be considered as the result of a lowered resistance of the islands through an hereditary tendency, in view of the rather frequent familial occurrences.

Sobolev concluded his report of his investigations by indicating how the internal secretion of the islands could be isolated in order to use it in the treatment of diabetes:

"By ligating the pancreatic duct," he wrote, "we now have a means of isolating the islands anatomically and of studying their chemical properties freed from the digestive ferments. This anatomical isolation will permit the testing, in a rational way, of an organotherapy for diabetes."

Convinced of the difficulty of obtaining enough glands with isolated islands, he even advised the use of newborn animals (calves) in which the islands are well developed in comparison with the acinar tissue which, moreover, is functioning on a very low level.

"We are justified in the hope that in the near future the question will be decided whether or not this method of approach will succeed in relieving the ills of the diabetic patient."

Obviously Sobolev had no opportunity to isolate the internal secretion himself, for in none of his papers does a hint in this direction appear. It remained for Banting and Best[9] to isolate insulin in 1921 in the same way that Sobolev had indicated. A paper covering part of the results of his investigations was read by Sobolev in January, 1900, at a meeting of the Association of Russian Physicians at St. Petersburg and was published in the same year in Russia and in Germany[10] as a preliminary communication. His complete thesis (177 pages) was published in the spring of 1901 in Russian, and a condensed version of thirty-three pages appeared in German.[11] The extensive list of references shows that Sobolev had read practically all the papers of importance which had appeared up to 1900 in English, French, German, Italian and Russian.

After receiving his degree, Sobolev applied for a travel fellowship. At the discussion of his candidacy for this grant, Pavlov stated that he was impressed by Sobolev's ability: "an eminent scientist, penetrating deeply into his problem." Sobolev was abroad for two years which he spent in part in Paris at the Pasteur Institute[12] (1902), and in part in Germany at several places including Marburg[13] (1903). In 1903 he returned to St. Petersburg where in 1904 he was appointed prosector and lecturer in the Department of Pathology.

In the same year he published another important paper.[13] He had attended, in the autumn of 1903, a demonstration course given by Professor Benda in Berlin. Benda demonstrated, among other things, the organs of a fifty-five-year-old woman who had died of diabetes and pulmonary tuberculosis. Sobolev received a piece of the caput, corpus and cauda pancreatis and in the section of the corpus observed an island of 1½ mm. diameter. This island he for various reasons considered rather as a compensatory hypertrophic and hyperplastic island than as an adenoma. Almost all the other islands were atrophic and a few showed hyaline degeneration. All changes were recorded in a set of drawings. The papers published by Opie[14,15] in 1901, the first to describe hyaline (now amyloid[16,17,18]) changes in the islands in diabetes, and by Nicholls,[19] who in 1902 described the first island cell adenoma, were both unknown to him. Sobolev was the first to report a macroscopically visible, circumscribed hypertrophic and hyperplastic island in the autopsy of a diabetic, and even now it remains most difficult to identify the borderline between hyperplasia and adenoma in those small structures.

In 1910 Sobolev[12] communicated lack of success in finding a substance which would cause diabetes by destruction of the islands. He conducted an extensive microscopical investigation of carcinoma and sarcoma of the pancreas, in which diseases the islands appeared to be the most resistant parts, surviving uninvolved even after tumor tissue surrounds them. This explained the absence of glucosuria. Thus once again microscopical examination revealed that whenever the pancreas is diseased but diabetes is absent, the islands remain relatively free from involvement. In those cases in which the Wirsungian duct was occluded by carcinoma a regeneration of island tissue from the smaller ducts was observed in the tail of the pancreas. Sobolev also con-

sidered the possibility of the existence of an island cell carcinoma; this would be a rarity, and could not originate from prenatally formed islands but from island cells which were later derived from duct epithelium.

In 1912, Sobolev[20] returned to his favorite subject for the last time. On morphological grounds he thought that regeneration of islands from smaller branches of the main duct, as it is to be seen in diabetes, leads to insufficiently differentiated and thus functionally inferior island tissue. Sobolev explained thus the presence of oversized islands in diabetes. These are unable, because of their inferiority, to replace diseased island tissue.

Ill health forced Sobolev to resign in 1912 and in 1919 he died, only forty-three years old, in the clinic for mental diseases in St. Petersburg. He was a gifted teacher who was interested in his students and a remarkable scientist who had a strong feeling for the technical side of his profession. He published in all, on various subjects, about forty papers, twelve of them in German. During his entire working life he gave a large share of his time to the study of the islands of Langerhans. His investigations were in many respects pioneering, and his accomplishments great by any standard.

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ABSTRACTS

Allwood, M. J.; Ginsburg, Jean; and Paton, A. (Sherington Sch. of Physiol., St. Thomas's Hosp. Medical Sch., London S.E. 1, England): THE EFFECT OF INSULIN HYPOGLYCAEMIA ON BLOOD FLOW IN INTACT AND SYMPATHECTOMIZED EXTREMITIES IN MAN. J. Physiol. *139*:97-107, Nov. 14, 1957.

Blood flow was measured in the limbs of seventeen intact and fifteen sympathectomized human subjects during insulin hypoglycemia, using venous occlusion plethysmography. In 'skin' segments—hand and foot—there was a variable response to hypoglycemia in intact limbs. A vasoconstriction occurred in sympathectomized limbs. In 'muscular' segments—fore-arm and calf—there was an increase in flow during hypoglycemia in both intact and sympathectomized limbs. The onset of flow changes following intravenous insulin was retarded by the infusion of a glucose-saline solution. Flow changes were absent in three subjects who failed to develop clinical evidence of hypoglycemia.

The variable response in the skin of the intact limb is believed to result from the constrictor response to circulating adrenalin being opposed by a dilatation mediated by the sympathetic nerve supply. It is suggested that the increased vasoconstrictor response seen in the sympathectomized

hand during hypoglycemia is not entirely due to supersensitivity of denervated vessels, but in part to loss of the dilatation normally present in the intact limb.

Batts, Adrienne A.; Bennett, Leslie L.; Ellis, Stanley; and George, Robert A. (Dept. of Physiol. and the Inst. of Exper. Biol., University of California, Berkeley, Calif.): GROWTH HORMONE-INDUCED REDUCTION OF GLYCOSURIA AND PARTIAL REPAIR OF THE ISLETS OF LANGERHANS IN PARTIALLY PANCREATECTOMIZED DIABETIC RATS. Endocrinology 59:620-30, December 1956.

Use of growth hormone on partially pancreatectomized diabetic rats showed an increase in glycosuria below the pre-injection levels; glycosuria remained reduced until one or two days after growth hormone was stopped. Histologically there was also improvement in the condition of the islet cells during hormone administration. These changes are interpreted as suggesting that growth hormone permits the beta cells to hypertrophy more effectively in response to the stimulus of an elevated blood sugar.

Beekman, Bruce E. (Zoology Dept., Indiana University, Bloomington, Ind.): THE EFFECT OF SYNTHALIN A ON BLOOD SUGAR AND PANCREATIC ALPHA ISLET CELLS OF THE FOWL. Endocrinology 59:708-12, December 1956.

A report of the cytotoxic effect of Synthalin A on the alpha islet cells in the fowl. Results were comparable to those in mammals except the initial hyperglycemia and the following hypoglycemia occurred more rapidly in the fowl.

Bennett, Claude E. (Letterman Army Hosp., San Francisco, Calif., now at 45th Field Hosp., APO 221, New York, N.Y.): CONGENITAL GALACTOSEMIA. U. S. Armed Forces M. J. 9: 112-19, January 1958.

The author reports a case of congenital galactosemia and emphasizes the urgent necessity for early diagnosis and appropriate treatment. Infants put on a galactose-free diet before the age of fifteen months developed normally, whereas those not treated or treated too late suffered serious injury, including liver damage, cataracts, and mental retardation. The metabolic defect in this disorder is discussed.

Berkman, James (Long Island Jewish Hosp., New Hyde Park, Long Island, N. Y.): THE KIMMELSTIEL-WILSON SYNDROME: PATHOLOGICAL CONSIDERATIONS. J. Mt. Sinai Hosp. 23:671-73, September-October 1956.

While the clinical and morphological features of the renal vascular lesions in diabetes have become well recognized, much remains to be known about the pathogenesis of the glomerular capillary lesion. Certain data pertinent to this question have been uncovered. The argyrophilic, trypsin-resistant material that composes the fully developed nodular lesion has the staining characteristics of collagen, sometimes contains sudanophilic fat, and is rich in carbohydrate. It has a specific ultraviolet absorption pattern. The application of recent advances in histochemical and microscopic technics seems to favor the initial localization of this material to the capillary wall rather than to an intercapillary space, but has not resolved this question. The axial distribution of nodular lesions is striking and constant and must have histogenetic implications, which in the present state of knowledge are best related to the existence of an axial space.

Burt, Richard L. (Dept. of Obstetrics and Gynecology, Bowman Gray Sch. of Med., Wake Forest Coll., Winston-Salem, N. C.): CARBOHYDRATE METABOLISM IN PREGNANCY: OBSERVATIONS ON GLUCAGON (HYPERGLYCEMIC-GLYCOGENOLYTIC FACTOR) IN NORMAL PREGNANCY AND THE PUERPERIUM. Am. J. Obst. & Gynec. 74:551-58, September 1957.

The hyperglycemic response to glucagon was studied in pregnant, postpartum, and nonpregnant subjects. Comparable hyperglycemia was found for each group. It is believed that glycogen mobilization by glucagon is unaffected by pregnancy. Attenuation of falls in inorganic phosphorus in late pregnancy and the early puerperium, associated with the hyperglycemia as experimentally induced, is interpreted as reflecting a gestational change in the peripheral utilization of glucose.

Campos, Paulo C.; and Namin, Ernesto P. (Dept. of Medicine, Coll. of Medicine, Univ. of the Philippines, Manila, P. I.): CLINICAL EXPERIENCE WITH HYPOGLYCEMIC SULFONAMIDES. J. Philippine M. A. 33:359-68, May 1957.

A clinical study of the effectiveness of two sulfonylureas, carbutamide and tolbutamide (Rastinon), in sixteen unselected diabetic patients. Response was better the oider the patient, the later the onset of the disease, the greater the patient's weight, and the longer the duration of the illness.

Caren, Raymond; and Corbo, Lucille (Sch. of Medicine, and Inst. for Med. Research, Cedars of Lebanon Hosp., Los Angeles, Calif.): PYRIMIDINE METABOLISM IN DIABETES MELLITUS STUDIED WITH THE URACIL TOLERANCE TEST. J. Clin. Endocrinol. 17:1071-80, September 1957.

There is no alteration in pyrimidine metabolism characteristic of diabetes mellitus, as measured by the uracil tolerance test. Abnormal uracil tolerance curves are frequent, both in patients with diabetes and those with other diseases.

Cotlar, Nathan (Baylor Univ. Coll. of Med., Houston, Tex.): COMA AND PSYCHOSIS FOLLOWING POSSIBLE INSULIN HYPERSENSITIVITY, WITH REMISSION OF NARCOTIC ADDICTION AFTER SEVERE HYPOGLYCEMIC SHOCK. Texas S. J. Med. 53: 860-62, November 1957.

Prolonged insulin shock, insulin hypersensitivity, organic brain disease in prolonged hypoglycemia, manic psychosis complicating recovery and an incidental cure of narcotic dependency as a result of the therapeutic effect of coma are noted in a case of a diabetic housewife admitted to the hospital in shock.

Dickinson, Lewis (Glasgow, Ky.): PROTEIN METABOLISM IN DIABETES MELLITUS AND VASCULAR DISEASE. J. Kentucky M. A. 55:894-97, October 1957.

The author has discussed protein metabolism and its alterations in diabetes mellitus. Some experimental reports on vascular disease in general have been reviewed, and an attempt has been made to correlate this material to support the hypothesis that vascular complications in diabetes mellitus are a result of chronic or intermittent protein deficiency due to excessive glyconeogenesis. The protein deficiency may be one of quality rather than quantity. It may be mild and consist only of deficiency of sulfur-containing or other essential amino acids. The evidence is highly circumstantial, but not conclusive.

Frawley, Thomas F. (National Inst. of Arthritis and Metabolic Diseases, National Inst. of Health, Bethesda, Md.): THE INTRAVENOUS USE OF SODIUM TOLBUTAMIDE IN ACUTE STUDIES. J. Clin. Endocrinol. *17*:1124-27, September 1957.

Once a subject's hypoglycemic dose is established with sodium tolbutamide it may be used in subsequent studies in which reproducible hypoglycemic effects are desired. Measurements of blood levels of tolbutamide have shown that the material is rapidly excreted or metabolized and that only negligible amounts remain in the blood after twenty-four hours. Therefore, studies can be repeated at about forty-eight-hour intervals without serious concern as to overlapping effects. With the use of intravenous sodium tolbutamide, it has been possible to perform a variety of acute metabolic and endocrine studies in both normal and diseased subjects. For instance, results of intravenous insulin tolerance tests, insulin-glucose tolerance tests, glucose-insulin tolerance tests and measurements of rate of disappearance of infused glucose have been compared with results of the same test procedures using sodium tolbutamide instead of insulin (e.g., intravenous tolbutamide tolerance tests, and tolbutamide-glucose tolerance tests). Intravenous sodium tolbutamide has been found useful in studies comparing the effect of insulin with that of tolbutamide on the volume of distribution of the so-called "insulin-responsive" pentoses, L-arabinose and D-xylose. The acute effects of tolbutamide on adrenocortical function have also been determined and found not to be significant.

Friedman, George Alexander (New York, N. Y.): DIABETES AND THE LAW. M. Times *85*:1039-46, September 1957.

Diabetes ranks eighth as a cause of death in this country. Failure to administer insulin to a diabetic patient prior to and immediately after an operation amounts to malpractice. Where a slight injury takes an unusually long time to heal, failure to inquire whether anything in the patient's history prevents it from healing properly, and more particularly, whether the patient suffers from diabetes, may amount to malpractice. Trauma is almost never the primary cause of diabetes. In medical opinion the time interval must be short, a matter of a few weeks, if the trauma and the disease are to be associated. In accident insurance cases there is no recovery when death is due to the contributing factors of accident and diabetes. Accident alone must be the cause of injury or death in order for the insured to recover. A diabetic insured cannot recover under a disability policy where he refused to submit to insulin treatment.

Garland, Hugh (Dept. of Neurology, General Infirmary, Leeds and Pinderfields Hosp., Wakefield, England): PANCREATIC ISLET ADENOMATOSIS WITH HYPOGLYCAEMIC EPISODES. Brit. M. J. *2*:969-71, Oct. 26, 1957.

The author reports a case of pancreatic islet adenomatosis that was allegedly the second in Great Britain and the eighth in the world to be described. The patient had a seven-year history of recurrent episodes of disturbed consciousness with amnesia and automatism reminiscent of the symptoms of temporal lobe dysfunction. Attacks did not occur in the fasting state, and the fasting blood sugar levels were never below 60 mg. per cent. The author suggests that when a diagnosis of organic hyperinsulinism is established, a hemipancreatectomy should be carried out if the pancreas appears to be normal at operation.

Given, William P.; and Tolstoi, Edward (Cornell Univ. Med. Coll., New York, N. Y.): PRESENT-DAY MANAGEMENT OF THE PREGNANT DIABETIC: SUCCESS OR FAILURE? S. Clin. North America *37*:369-78, April 1957.

The authors review their experience with 131 pregnancies in diabetics treated during the years 1932 to 1948, and with 113 cases treated during the period of 1949 to 1955. Patients in the former group were treated as uncomplicated pregnancies, while the latter were closely followed at frequent intervals according to currently accepted principles of the management of diabetic pregnancies. There was no appreciable difference of fetal mortality between the two groups. The authors conclude that the currently accepted principles of therapy are important, but that further research along endocrine and metabolic lines is necessary for a solution to the problem.

Gordon, Edgar S.: THE HAZARDS OF EXCESSIVE INSULIN DOSAGE. Wisconsin M. J. *56*:449-50, October 1957.

The author concludes that the occurrence of insulin-induced hypoglycemia, especially in young diabetic patients, should be considered to be far more serious than the simple inconvenience of the transient episode. It may be stated categorically as a basic principle that diabetic patients should have no insulin reactions and their insulin dosage should be adjusted accordingly. Similarly, the first step in management of the patient who is swinging from hypo- to hyperglycemia should always be to reduce the insulin dosage carefully and (probably) gradually until there are no hypoglycemic attacks, even though there may be considerable glycosuria at times. When such a procedure is instituted, the patient is allowed to spill glucose in the urine but is protected against heavy ketosis by very small supplementary doses of standard insulin if necessary.

Hauser, T. E.; and Flanagan, E. B. (Carlsbad, N. M.): PRELIMINARY REPORT ON EXPERIENCE WITH BZ-55 ON TEN DIABETICS. Southwestern Med. *38*:254-55, April 1957.

The authors report experience with BZ-55 on ten patients over a period of three months. Nine patients were able to discontinue insulin and remained controlled. The authors observed that BZ-55 appeared to work best in the elderly diabetic with a short history of diabetes.

Hendon, James Robert (Sect. on Endocrinology, Univ. of Louisville Sch. of Med., Louisville, Ky.): CLINICAL EXPERIENCE WITH ORAL HYPOGLYCEMIC SULFONYLUREAS. J. Kentucky M. A. *56*:29-32, January 1958.

The author concludes: The sulfonylureas have presented us with a new facet of treatment of diabetes mellitus. Whether their use constitutes an advance remains to be seen. In diabetes we meet with a mysterious metabolic blunder, and hyperglycemia and ketosis are its more dramatic manifestations. Whether the projected course of the disease will be altered by these hypoglycemic agents is unknown. In any case, it is imperative that the effectiveness of the sulfonylureas not be overrated, to the exclusion of other measures, for instance, weight control. If used in such a way, they can only bring disservice to our patients.

Hollifield, Guy; and Parson, William (Univ. of Virginia Sch. of Med., Charlottesville, Va.): FOOD DRIVE AND SATIETY IN YELLOW MICE. Am. J. Physiol. *189*:36-38, April 1957.

Spontaneous running activity during ad libitum feeding, fasting and refeeding was studied in inbred yellow mice.

These studies suggest that the yellow gene per se is not associated with reduced activity and that inbred yellow mice have intact hypothalamic feeding centers. These findings suggest that the obesity in yellow mice has a more complex etiology than reduction in activity and may be associated with an altered cellular metabolism especially in regard to fat.

Holten, Cai (Med. Univ. Dept., Aarhus Kommunehospital, Aarhus, Denmark): HYPOGLYCAEMIA-INDUCING TUMOUR RESEMBLING SPINDLE-CELL SARCOMA. Acta med. scandinav. *157*:97-102, March 25, 1957.

In a forty-one-year-old woman a large retroperitoneal tumor perforating the ascending colon and causing severe intestinal bleeding was found on operation in January 1947. Histological examination showed spindle-cell sarcoma. Five years after the removal of the primary tumor she developed severe hypoglycemic attacks. Tumor-metastases in the liver were present. For eighteen months low blood sugar values were repeatedly found, and several severe hypoglycemic attacks occurred. Post-mortem examination showed numerous liver metastases with a histological picture very similar to that of the primary tumor. On review a close resemblance of the histological picture to that in previously reported cases of Skillern et al., with large tumors simulating sarcoma and causing hypoglycemic attacks, was demonstrated.

House, E. L.; Nace, P. F.; and Tassoni, J. P. (Dept. of Anatomy, New York Med. Coll., New York, N. Y.): ALLOXAN DIABETES IN THE HAMSTER: ORGAN CHANGES DURING THE FIRST DAY. Endocrinology *59*:433-43, October 1956.

More than 200 hamsters were injected with alloxan and a histochemical study was made of kidneys, liver and pancreatic tissue during the first twenty-four hours. Results showed: (1) during initial hyperglycemia liver glycogen was almost completely lost; (2) β cells lost ability to synthesize insulin between two and five hours; (3) 95 per cent of β cells degenerated and were replaced by mononuclear cells between five and twenty-four hours; (4) no degenerative changes occurred in the kidney; (5) there was evidence of alloxan's initial beta cell stimulatory effect in the early increase in granulation of beta cells.

Houssay, B. A.; and Penhos, J. C. (Inst. of Biology and Experimental Med., Costa Rica 4185, Buenos Aires, Argentina): DIABETOGENIC ACTION OF PITUITARY HORMONES ON ADRENALECTOMIZED HYPOPHYSECTOMIZED DOGS. Endocrinology *59*:637-41, December 1956.

Three dogs were 82 to 85 per cent pancreatectomized, hypophysectomized and bilaterally adrenalectomized. Following this they were given somatotrophin, prolactin and ACTH and the levels of glycemia observed. Conclusions drawn were that somatotrophin and prolactin have a diabetogenic action in dogs deprived of hypophysis, adrenals and 85 per cent of pancreas, and thus hypophyses and adrenals are not essential for the production of the diabetogenic effect of these hormones. ACTH had no action on the glycemia.

Huggett, A. St. G.; and Nixon, D. A. (Prof. of Physiol., Univ. of London; Physiol. Dept., St. Mary's Hosp. Med. Sch., London, England): USE OF GLUCOSE OXIDASE, PEROXIDASE, AND O-DIANISIDINE IN DETERMINATION OF BLOOD AND URINARY GLUCOSE. Lancet *2*:368-70, Aug. 24, 1957.

A description of a method for routine measurement of blood and urinary glucose using a mixed enzyme-oxygen acceptor reagent. The reagent is simple to prepare, the method involves few operative steps and it measures only the true glucose level.

Jackson, W. P. U.; and Woolf, N. (Groote Schuur Hosp., and Univ. of Cape Town, Cape Town, South Africa): FURTHER STUDIES IN PREDIABETES. Lancet *1*:614-17, Mar. 23, 1957.

A report is made of sixteen women diagnosed as prediabetics during pregnancy who later became frankly diabetic. Clues used in detection of prediabetics were the birth of babies who were too large, too heavy, fat, edematous, weak or "Cushingoid"; a family history of diabetes; glycosuria during pregnancy; or repeated abortions. A glucose tolerance test was run on patients suspected of being prediabetics. The most common alteration in the test was an elevation in blood sugar values at two or two and one-half hours. All women diagnosed as prediabetic by glucose tolerance test developed a more definite abnormality after a follow-up period of at least three years.

Kigoshi, Shigeru (Dept. of Pharmacol., Res. Inst. of Tuberculosis, Kanazawa Univ., Kanazawa, Japan): ON THE TUBERCULIN-HYPOGLYCEMIA IN TUBERCULOSIS GUINEA PIGS. Jap. J. Tuberc. *4*:153-58, December 1956.

Blood sugar determination experiments on tuberculous guinea pigs receiving a lethal dose of tuberculin revealed that severe and prolonged hypoglycemia occurred in all these animals.

Kitamoto, Osamu; and Abe, Teitaro (Dept. of Clinical Res., Inst. for Infectious Diseases, Univ. of Tokyo, Tokyo, Japan): STUDIES ON THE SO-CALLED P.A.S. GLYCOSURIA AND DIFFERENTIAL DIAGNOSTIC METHOD. Jap. J. Tuberc. *4*:126-32, December 1956.

The authors concluded that the positive sugar reaction in the so-called P.A.S. glycosuria seemed to be due to glucuronic acid.

Kuusisto, A. N.; and Antila, Viljo (Wihuri Res. Inst. & Pharmaceutical Manufacturers Orion Oy, Helsinki, Finland): HAS THE NEW ANTIDIABETIC DRUG, N_1-SULPHANILYL-N_2-N-BUTYLCARBAMIDE, GOITROGENIC PROPERTIES? AN EXPERIMENTAL STUDY. Acta Endocrinol. *23*:433-36, December 1956.

The action of the new antidiabetic drug, N_1-sulphanilyl-N_2-n-butylcarbamide, was studied in the rat. It was found that the thyroid not only increased in weight in the treated animals but the thyroids also appeared to be histologically more active than in the controls. The serum protein-bound iodine was lower in the experimental animals than in the controls. It would seem that in the rat N_1-sulphanilyl-N_2-n-butylcarbamide exerts an effect similar to that of goitrogenic drugs.

Leschinskaja, I. S. (Sect. of Functional Diagn., Ukrainian Inst. of Clin. Med., Kiev, Russia): THE SIGNIFICANCE OF THE KETOSIS IN VARIOUS FUNCTIONAL TESTS FOR THE ASSESSMENT OF CLINICAL FORMS OF DIABETES MELLITUS. Probl. Endokr. *2*:8-14, 1956.

In severe forms of diabetes mellitus, insulin contributes to the lowering of ketonemia, but glucose by mouth generally does not affect the ketone level and in some cases may even increase it. Adrenalin causes a high level of ketonemia, with an insignificant lowering in two to three hours. In a number of cases with average and severe forms of diabetes, insulin, while lowering the blood sugar level, did not contribute to a decrease of the high concentration of ketones. In patients with a lessened sensitivity to insulin, administration of this prepara-

tion did not result in a sufficient decrease of glycemia; the blood ketone level did not fall either. When the level of glycemia fell abruptly two to three hours after insulin and also three to four hours after glucose by mouth, a rise in ketonemia was noticed. (Russian)

Lewis, Edward C., II; and Brown, Harvey E., Jr. (Veterans Admin. Hosp., Coral Gables, Fla.): AGAMMAGLOBULINEMIA ASSOCIATED WITH PERNICIOUS ANEMIA AND DIABETES MELLITUS. A. M. A. Arch. Int. Med. *100*:296-99, August 1957.

The diagnosis of agammaglobulinemia was made in a thirty-year-old white man with Addisonian (pernicious) anemia and diabetes mellitus. The history suggested the onset of the disease at about the age of eighteen years. The use of pooled human gamma globulin for maintenance, and antimicrobials for acute infections, has been satisfactory to date.

Mayer, Jean; and Vitale, Joseph J. (Harvard Sch. of Public Health, Boston, Mass.): THERMOCHEMICAL EFFICIENCY OF GROWTH IN RATS. Am. J. Physiol. *189*:39-42, April 1957.

A simultaneous study of changes in food intake and body composition during the three months of postweaning growth was undertaken on three large groups of albino rats fed semisynthetic diets containing, respectively, 10, 25 and 60 per cent protein. The results show a remarkable similarity of percentage of body weight represented by protein during the whole growth period for the three groups, as well as constancy within each group. The thermochemical efficiency (ratio of calories deposited to calories ingested) was constant from weaning to puberty and particularly so on the diet with the protein content most favorable for growth (25 per cent). The thermochemical efficiency ratio of calories deposited to calories ingested was approximately constant from weaning to puberty with optimal diets and high protein diets; it declined somewhat earlier with a low protein diet.

Mayes, P. A.; and Robson, W. (Dept. Biochem., King's Coll., Strand, London, W.C. 2, England): THE DETERMINATION OF KETONE BODIES. Biochem. J. *67*:11-15, September 1957.

The authors describe an accurate quantitative analytic method for ketone bodies in small samples (0.1 ml.) of blood in urine. The method is based on the conversion of all ketone bodies into acetone, on the reaction of acetone with 2:4-dinitrophenylhydrazine and on subsequent colorimetric determination of acetone-2:4-dinitrophenylhydrazone in carbon tetrachloride. A combined reflux and distillation apparatus has been designed which ensures reproducibility in the estimation of β-hydroxybutyric acid and allows the separate and direct estimation of acetone plus acetoacetic acid and of β-hydroxybutyric acid from one sample. At a ketonemic level of 40 mg./100 ml., the standard deviation of an individual reading is less than ±0.2 mg./100 ml.

Mirsky, I. Arthur; Diengott, Daniel; and Perisutti, Gladys (Dept. Clinical Science, Univ. of Pittsburgh Sch. of Med., Pittsburgh, Pa.): THE HYPOGLYCEMIC AND INSULINASE-INHIBITORY ACTION OF SOME PLANT GROWTH REGULATORS. Endocrinology *59*:715-18, December 1956.

A report of experiments demonstrating that the administration of plant growth hormones (indoleacetic acid, indolepropionic acid, indolebutyric acid and p-chlorphenoxyacetic acid) orally to rats results in a decrease in the blood sugar concentration and an associated decrease in insulinase activity. The hypoglycemic response seems to be related to a concomitant decrease in insulinase activity.

Mohnike, G. (Diabetikerheim, Karlsburg, Kreis Greifswald, Germany): ON DIABETIC VASCULAR DISEASE. Deutsche med. Wchnschr. *82*:1904-08, Nov. 8, 1957.

The frequency and pathogenesis of diabetic angiopathies were studied in 2,600 hospitalized and 3,000 ambulatory patients. Only the specific diabetic angiopathies were considered that affect the capillaries and the neighboring parts of the arterioles and venules, with a predilection for the vessels of the kidney and the eye, and that are characterized by the deposition of polysaccharides or mucopolysaccharide complexes. Statistical evaluation showed that diabetic angiopathies occur rarely earlier than five years after the onset of clinical diabetes. Their incidence correlated well with both the duration and the severity of the diabetes. Thus retinopathies were found in about 10 to 15 per cent of the diabetics of various ages with a duration of the disease of zero to five years, while the incidence was 40 to 60 per cent in the various age groups with a duration of the disease of six to fifteen years. While in this latter group the incidence was the highest in patients with onset of the disease in early life, retinopathy was more common in the older patients of the group with diabetes of recent onset. Nephropathies had their highest incidence (28 per cent) among the diabetics with childhood onset of the disease and a duration of six to fifteen years. In the group with diabetes of less than five years' duration, nephropathies were more frequent among the elderly patients (15 per cent). Poor control of the condition seemed to favor the occurrence of angiopathies, although good control could not prevent their occurrence in diabetes of long standing. Thus in the group of well-controlled disease with a duration of fifteen years or longer, the incidence of angiopathies was 30 per cent as compared with 60 per cent and 80 per cent in the groups under fair and under poor control with duration of the disease of comparable length. A far smaller incidence of the complication was found in the thin and the hyperthyroid diabetic; if angiopathies occur at all in this group, they run a more benign course, although the diabetic control is more difficult. Retinopathies were found to be particularly frequent and malignant in juvenile diabetes with retarded sexual development or with hyperfunction of the adrenals. Observations on 150 pregnant diabetics, on the other hand, indicated that pregnancy has no specific harmful effects, unless vascular damage is pre-existent. In regard to treatment, the author emphasizes the important prophylactic role of prevention of overweight with a low fat, low calorie diet. He advises keeping insulin at its minimal required dose; insulin overdosage is considered harmful.

Morandi, L.; Essellier, A. F.; and Jeanneret, P. (Dept. of Med., Univ. of Zurich, Zurich, Switzerland): THE MECHANISM OF INSULIN—EOSINOPENIA. Klin. Wchnschr. *35*:1019-22, Oct. 15, 1957.

The role of the hormones of the anterior pituitary and the adrenals in the development of insulin eosinopenia was examined in patients who had undergone either hypophysectomy or adrenalectomy (because of metastatic breast carcinoma). Insulin tests (0.1 U/kg. i.v.), combined insulin-cortisone tests (25 mg. cortisone orally two hours prior to the administration of insulin) and cortisone tests were carried out. The blood sugar was determined at the usual intervals over a two-hour

period, the eosinophile count was done at two-hour intervals for a period of six hours. After hypophysectomy, insulin hypoglycemia was not accompanied by eosinopenia, but the combined insulin-cortisone test yielded eosinopenia. After adrenalectomy only the combined insulin-cortisone test was done and failed to cause eosinopenia although significant hypoglycemia was obtained. The authors concluded from these experiments that an intact pituitary-adrenal system is necessary for the insulin eosinopenia. The eosinopenia is not due to insulin itself but to the insulin-induced hypoglycemia, which calls forward an adrenalin release and, if severe enough, also a release of ACTH and glucocorticoids. Adrenalin increases the eosinopenic action of the circulating glucocorticoids.

Mosca, L. (Dept. of Pathol. Anat., Univ. of Pavia, Italy): AN EXPERIMENTAL STUDY OF THE CYTOLOGY OF PANCREATIC ISLETS. Quart. J. Exper. Physiol. *42*:49-55, January 1957.

The pancreatic islet apparatus of all vertebrates is mainly composed of alpha and beta cells. A third type of cell is sometimes visible among alpha cells, especially in fish. By means of different treatments (anoxia, cobalt chloride or insulin injections) it is possible to observe that both alpha and beta cells can be transformed into the third type. It is concluded that this type of cell is only a functional stage either of alpha or of beta cells.

Nikiforova, N. I. (2nd Med. Inst., Moscow, Russia): THE ABDOMINAL SYNDROME IN DIABETIC COMA IN CHILDREN. Probl. Endokr. *1*:37-40, 1955.

The development of the peritoneal syndrome during a diabetic coma is described in a patient thirteen years of age. (Russian)

Plattner, H. C.; and Scherer, J. (Clinique Universitaire de Medecine Interne de Geneve, Service G., Bickel., Geneva, Switzerland): STUDIES OF THE MODE OF ACTION OF THE SULFONYLUREAS IN THE HUMAN DIABETIC SUBJECT. Rev. franç. études clin. et biol. *2*:803-07, October 1957.

In studying the hypoglycemic action of the sulfonylureas the authors repeated carbohydrate tolerance tests on three occasions in fourteen patients with benign diabetes. The first test was a simple glucose tolerance after three days without insulin on a standard 200 gm. carbohydrate diet. The second test was done after the administration of 100 mg. cortisone. The third test was again with cortisone but after three days' treatment with sulfonylurea (BZ-55). The mean values for the tolerance test after cortisone were significantly higher than in the first test. The mean values for the third test (with cortisone but after sulfonylurea) were again comparable with the first test. The results were interpreted as suggesting that there is an antagonism between the hyperglycemic action of glucocorticoids and the hypoglycemic effect of the sulfonylureas.

Pomeranze, Julius (New York Med. Coll., Metropolitan Med. Center, New York, N. Y.): THE TREATMENT OF SYMPATHOTONIA IN LABILE DIABETES MELLITUS. New York J. Med. 57:3326-28, Oct. 15, 1957.

Labile diabetes is in part the result of the too vigorous application of insulin in the presence of hyperglycemia and mild ketonemia. Labile diabetes may be related to anxiety and sympathotonia when all other causes are absent. Removal of anxiety by a careful program of reassurance and emotional support, aided with a carefully considered dosage of Rauwolfia preparations, frequently creates a stable, smoothly controlled young diabetic patient. The therapeutic dose of one such Rauwolfia preparation (Raudixin) appears to be less than that causing cumulative side effects. There is a wide variability of response in individuals, which may be due in part to variations in intestinal absorption. The protective role of the physician cannot be replaced with tranquilizing drugs.

Ranke, Eugene J. (Dept. of Med., Univ. of Illinois, Chicago, Ill.): THE OFFICE MANAGEMENT OF DIABETES MELLITUS. GP *15*:85-90, June 1957.

Candidates for dietary management with or without insulin were grouped according to the level of the fasting blood sugar and fractional urinalysis obtained before meals and at bedtime. Insulin is indicated in that patient on dietary management who is unable to regain previously lost weight, who develops undesired weight loss, who has poor general health or who is unable to combat satisfactorily the complications of diabetes.

Refresher Article. INFANTS BORN OF DIABETIC AND PREDIABETIC MOTHERS. M. Times *85*:951-55, September 1957.

The infant of the diabetic and prediabetic presents a clinical picture that may be due to hormonal imbalance. The distress and death of the neonate may be due to electrolytic imbalance secondary to this same hormonal imbalance, anoxia, or pulmonary pathology. The prognosis for these infants is generally good; however, a good percentage develop juvenile diabetes.

Reid, James; Macdougall, A. I.; and Andrews, M. M. (Med. Research Council, Western Infirmary, Glasgow, Scotland): ASPIRIN AND DIABETES MELLITUS. Brit. M. J. *2*:1071-74, Nov. 9, 1957.

The authors report the case of a young diabetic in whom glycosuria and normal fasting blood sugar occurred during aspirin therapy for acute rheumatism. An additional seven mild to moderately severe diabetics were also given an intensive two-week course of large doses of aspirin, with resulting abolition of glycosuria and lowering of the fasting blood sugar to normal or nearly normal values. There was no decisive effect on glucose tolerance. Moderate ketonuria in two patients was reduced to normal with aspirin. Clinical improvement accompanied the biochemical changes induced by aspirin and, while serious toxic manifestations were not conspicuous, tinnitus and deafness were annoying. The possible place of aspirin in the treatment of diabetes mellitus is discussed.

The effects of the aspirin on food intake were not considered in this report.

Schimek, Robert A. (Dept. of Ophth., Henry Ford Hosp., Detroit, Mich.): HYPOPHYSECTOMY FOR DIABETIC RETINOPATHY: A PRELIMINARY REPORT. A.M.A. Arch. Ophth. *56*: 416-25, September 1956.

The procedure, experimental and unproved in value, was carried out in five cases of severe diabetic retinopathy. The patients were followed from six months to well over a year. There was no mortality. Three patients showed no further progression of their retinopathy. Another patient showed improvement in one eye and deterioration in the other. The other patient has continued to show improvement in his vision. In

addition to changes in the course of the retinopathy, the patients had a lessening of insulin requirements, drop in blood pressure and diminished function of the gonads, thyroid and adrenal glands.

Scholz, Donald A.; Woolner, Lewis B.; and Priestley, James T. (Mayo Clin. and Mayo Found., Rochester, Minn.): SPONTANEOUS HYPOGLYCEMIA ASSOCIATED WITH FIBROGENIC TUMOR: REPORT OF TWO CASES. Ann. Int. Med. *46*:796-807, April 1957.

Authors report the tenth and eleventh cases of fibrogenic tumors whose presenting symptoms were due to recurrent hypoglycemia. In one case surgical removal of a fibrosarcoma of the kidney produced complete relief of the symptoms. In the other case there was a poorly differentiated fibrosarcoma of the liver in a patient who later died from his malignancy. There was no islet cell tumor of the pancreas. Fibrogenic tumors associated with hypoglycemic symptoms are usually in the retroperitoneal region, but may also be found in the thorax.

Schreus, H. Th.; and Ippen, H. (Dept. of Dermatology, Med. Acad., Düsseldorf, Germany): PHOTO-ALLERGY CAUSED BY AN ORAL ANTIDIABETIC DRUG. Deutsche med. Wchnschr. *83*: 98-100, Jan. 17, 1958.

A seventy-year-old diabetic female was placed on BZ-55 (250 mg./day). Her mild diabetes responded well. Soon after onset of this therapy she developed eczematous eruptions on the face, hands and legs. The face was markedly swollen and reddish; blisters and erosions developed on the affected skin areas. BZ-55 was discontinued after a total intake of not more than ten tablets, or 5 gm. of carbutamide. The skin eruptions disappeared rapidly. Special examinations with exposure to radiation of different frequency spectra demonstrated that this reaction represented a photo-allergy. The authors suggest that this photo-allergy to carbutamide develops in two stages. The first consists of the sulfonamide combining with a protein to form an antigen precursor which absorbs sunlight. The second consists of the conversion of the precursor into the full antigen by the energy of the absorbed light.

Schwarz, V.; Holzel, A.; and Komrower, G. M. (Univ. of Manchester, Manchester, England): LABORATORY DIAGNOSIS OF CONGENITAL GALACTOSAEMIA AT BIRTH. Lancet *1*: 24-25, Jan. 4, 1958.

The authors describe a case of congenital galactosemia in which the diagnosis was established within a few days of birth by examination of umbilical cord blood. Infants with congenital galactosemia can be given, from the first, a galactose-free diet and can thus be saved from the damaging effect of extraneous galactose. The method for examining cord blood to determine the galactose content is described.

Singh, Inder (Lieutenant-Colonel, A.M.C.): ORAL TREATMENT OF DIABETES WITH TOLBUTAMIDE. Brit. M. J. *2*:1345-47, Dec. 7, 1957.

The author describes the results of tolbutamide treatment in 136 cases of "adult-onset" in diabetes mellitus. The results were very satisfactory in ninety-four cases (68 per cent), satisfactory in thirty-two (23 per cent), and there were eleven failures (9 per cent). The result was also satisfactory in one case of congenital diabetes in a child four years of age. No serious side reactions were encountered. The factors influencing the action of the drug, and indications for its use, are discussed.

Skensved, Ole (Med. Dept. of Statshospitalet, Sønderborg, Denmark): STUDIES ON A NEW LONG-ACTING INSULIN: ZINC METHYL-ALBUMIN INSULIN. Acta Endocrinol. *24*:159-78, February 1957.

Insulin is freely soluble at the *p*H of the blood and is therefore absorbed within a few hours. In order to obtain a retarded absorption and consequently a prolongation of the therapeutic effect over a period of up to twenty-four hours, the insulin must be slowly liberated at *p*H 7. For twenty years only the naturally occurring alkaline proteins have been used to obtain depot action. In fact they have been believed to be the only available media that were not at the same time antigenic. But this is not so. Human albumin is a nonantigenic, well-defined substance. Its reaction is acid, but a methylation process blocks the acid groups, so that the methyl albumin behaves as a basic protein. Accordingly, it can become bound to the acid insulin, thus forming an insulin preparation slightly soluble at *p*H 7. The use of human albumin is attractive, and the incidence of allergic reactions to this preparation might be expected to be reduced compared with previous retardation media. The preparation was tested clinically in a series of twenty-two patients consisting of seven men and fifteen women, ranging in age from seventeen to seventy-five years, with an average of about fifty-one years. The duration of diabetes ranged from one month to sixteen years, treatment with insulin in fifteen cases lasting from a few weeks to sixteen years. Among fifteen patients who had previously been receiving insulin the following findings were made on re-adjustment to ZMAI: eight exhibited a reduction in insulin requirement of from 36 to 4 units, averaging 17.5 units. In three cases the requirement remained unchanged, and in four cases it increased by 4 units per patient. On the basis of present findings it is concluded that ZMAI is in advance of other preparations with regard to the manner in which its depot effect is achieved, i.e., by using human albumin. Moreover, its clinical effect in controlling diabetes appears to be equal to that of other long-acting insulins, and in this series often better, and rarely poorer. It is also notable that its clinical use does not appear to give rise to severe untoward reactions.

Sodhi, Harbhajan S. (New York, N. Y.): DIABETES MELLITUS, INSULIN AND GLUCAGON. M. Times *85*:1013-19, September 1957.

The author concludes: Recent studies suggest that it is possible to augment the effect of insulin on peripheral glucose utilization with glucagon in some diabetic patients.

Srinivasan, M. (Central Food Technological Res. Inst., Mysore, India): EFFECTS OF CERTAIN PROTEIN FOODS ON BLOOD-SUGAR LEVELS AND GLUCOSE TOLERANCE. Lancet *2*:317-20, Aug. 17, 1957.

A report of the effects of certain protein foods on blood sugar levels and glucose tolerance tests in three diabetic men and two normal men. The study showed that certain protein foods depressed the peak of blood sugars following administration of glucose in both the diabetic and normal men. Best results at the arbitrary levels of protein ingested were derived from casein and protein from black grain. The possibility

of controlling hyperglycemia by qualitative rather than quantitative feedings is discussed.

Stetten, DeWitt, Jr.: EDITORIAL: THE HYPOGLYCEMIC SULFONYLUREA DRUGS—AN INTERIM EVALUATION. Ann. Int. Med. *46*:1005-08, May 1957.

Theoretical considerations are discussed and caution is advised in determining the place and value of the sulfonylurea drugs in the treatment of diabetes.

Volk, David (Western Reserve Univ. Sch. of Med., Cleveland, Ohio): DISSIMILARITY OF RETINAL MICRO-ANEURYSM AND GLOMERULAR NODULE IN DIABETES. A.M.A. Arch. Ophth. 56:188-93, August 1956.

The retinopathy characteristic of diabetes with its specific microaneurysm seen at the points of bifurcation, or at the loops in the capillary, is described. The renal nodule usually at the periphery of the glomerulus appeared as a localized mass of dense collagenous tissue which was hyalinized. It is pointed out that in Ashton's post-mortem material retinopathy was much more frequent than diabetic nephropathy but the latter did not occur in the absence of diabetic retinopathy. The dissimilarity of the two lesions is suggested in the mode of development. The hyalinized retinal microaneurysm develops first as a microaneurysm; the renal nodule is the result of progressive proliferation of connective tissue about normal sized or dilated glomerular capillaries. Though capillary budding occurs in the retina, it has not been demonstrated in the kidney. Hemorrhages likewise appear confined to the retina. The retinopathy of Kimmelstiel-Wilson syndrome takes on the characteristics of hypertensive-arteriosclerotic retinopathy.

Williams, Robert H.; Henley, Elaine D. (University of Washington, School of Med.): RECENT STUDIES RELATIVE TO THE TREATMENT OF DIABETES: SPECIAL REFERENCE TO NEW ORAL ANTIDIABETIC DRUGS. A. M. A. Arch. Int. Med. 99: 501-15, April 1957.

An excellent discussion of the pathogenesis of diabetes and the various therapies for the disease. Special attention is given to the use of carbutamide and tolbutamide. Fifty per cent of the patients show a relatively good response to sulfonylureas. Less than 10 per cent of the juvenile diabetics respond while more than 80 per cent of those over sixty respond well.

Williams, Robert H.; Tyberghein, Jean M.; Hyde, Paul M.; and Nielsen, Robert L. (Dept. of Medicine, University of Washington Sch. of Medicine, Seattle, Wash.): STUDIES RELATED TO THE HYPOGLYCEMIC ACTION OF PHENETHYLDIGUANIDE. Metabolism *6*:311-19, July 1957.

The authors present evidence indicating that the hypoglycemia induced by phenethyldiguanide may be caused by increased anaerobic glycolysis. In vitro studies with muscle demonstrate an increased glucose uptake, decreased glycogen storage and increased lactic acid production. There is evidence to suggest a decrease in gluconeogenesis in vivo. The studies were made upon rats and guinea pigs, the muscle studies were hemidiaphragms from rats and guinea pig liver homogenate. Many of the effects observed by phenethyldiguanide have been previously observed with decamethylene diguanide (Synthalin A). Toxic effects have been less intense with phenethyldiguanide.

Wolfe, Frederick (Postgrad. Med. Sch. of London and the Wellcome Foundation, London, England): DOSAGE OF TOLBUTAMIDE. Lancet *2*:191, July 27, 1957.

The results of administration of tolbutamide are presented. The majority of the patients were managed on a 1½-2 gm./day.

Wolff, Herman J.; Kennedy, B. J.; and Johnson, Morris B. (Minneapolis, and St. Paul, Minn.): EFFECT OF BILATERAL ADRENALECTOMY ON DIABETES MELLITUS. Minnesota Med. *40*:318-21, May 1957.

At least twelve cases of total bilateral adrenalectomy in diabetic patients with advanced vascular disease have now been reported, and there are certainly others not yet documented. Of these twelve patients, five died postoperatively due either to adrenal insufficiency (three) or to continued progression of the degenerative vascular lesions (two). It appears that at least one reason for the unfavorable outcome of adrenalectomy in this series of cases lies in the advanced degree of vascular deterioration that always has been present before operation was considered. Consideration of total bilateral adrenalectomy as a therapeutic measure in the initial stages of arteriolar degeneration would appear to represent radical therapy, but it is evident that procrastination until the vascular or renal lesions have reached the stage of irreversibility has not been successful. As an ancillary procedure calculated to prolong life in a diabetic patient with advanced vascular disease, total adrenalectomy does not appear to deserve approbation.

Wright, J. T. (Buckhurst Hill, Essex, England): TREATMENT OF A DIABETIC DOG. Lancet *1*:1201-02, June 8, 1957.

The author relates the trials and tribulations in the treatment of diabetic dogs, his own canine pet in particular. The dog apparently died in hypoglycemic shock, and autopsy examination failed to reveal any macroscopic evidence of pancreatic disease. The microscopic examination was not reliable because of post-mortem changes.

Yankelevich, D. E. (Dept. of Pathophysiol. of the Ukrainian Inst. of Endocrinology, Kharkov, Russia): THE INFLUENCE OF THE HIGHER CENTRES OF THE CENTRAL NERVOUS SYSTEM ON THE REACTION OF THE ORGANISM TO INSULIN. Probl. Endokr. *1*:75-80, 1955.

Prolonged administration (up to two years) to dogs of 0.25-0.5 units insulin per kg. body weight was followed by resistance to the hormone. After two months, complete resistance to insulin was created, but later recovery of sensitivity was observed in one of two animals. (Russian)

Ziegler, Dewey K.; and Presthus, Jan (Univ. of Minnesota Med. Sch., Minneapolis, Minn.): NORMAL ELECTROENCEPHALOGRAM AT DEEP LEVELS OF HYPOGLYCEMIA. Electroencephalog. & Clin. Neurophysiol. 9:523-26, August 1957.

Intravenous insulin was given to a series of twenty-four young adult patients, with diagnoses of syncopal episodes or atypical seizures, in an attempt to elicit EEG abnormalities. Hypoglycemia of extreme degree was achieved thirty minutes after injection in almost all patients. In thirteen patients the EEG remained normal at the depth of hypoglycemia; in nine of these it also was normal after three minutes of hyperventilation during hypoglycemia.

ORGANIZATION SECTION

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HENRY T. RICKETTS, M.D., *Chicago;* FREDERICK W. WILLIAMS, M.D., *New York*

Program of the 18th Annual Meeting of the American Diabetes Association June 21-22, Hotel Mark Hopkins, San Francisco, California

"The Prediabetic State in Man: Definition, Interpretation and Implications," is the title of the Banting Memorial Lecture of the American Diabetes Association which will be delivered by Jerome W. Conn, M.D., at the Eighteenth Annual Meeting. The Lecture will be given from 11 a.m.—12 noon during the Scientific Session, Saturday morning, June 21. Dr. Conn is Professor of Medicine and Director of the Metabolism and Research Unit, University of Michigan Medical School; and Chief, Department of Endocrinology and Metabolism, University Hospital, Ann Arbor, Michigan. He will be presented with the Banting Medal at the Annual Banquet of the Association to be held that evening. Also the 1958 Lilly award will be presented at the Banquet to James B. Field, M.D., of the National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland. The Annual Award, supported by Eli Lilly and Company, consists of $1,000 and a medal. Its purpose is to recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality. John A. Reed, M.D., President of the American Diabetes Association, will deliver the Presidential Address. The principal speaker will be Mr. William H. Thompson, Jr., of the Union Oil Company of California.

Organizational meetings which have been scheduled for Saturday, June 21, include: Luncheon for Lay Delegates, 12:30 p.m.—1:30 p.m.; Assembly of Delegates, 1:30 p.m.—3:00 p.m.; Board of State Governors, 3:30 p.m.—5:00 p.m.; Special Program for Lay Delegates, 3:30 p.m.—5:00 p.m.

Although a Joint Meeting per se will not be held with The Endocrine Society, arrangements have been made so that each organization will mutually recognize the badges of the other. Endocrine Society members therefore may attend the Scientific Sessions of the American Diabetes Association and ADA members may attend sessions on diabetes of The Endocrine Society without charge by presenting their respective registration badges at the time of admission. The program of The Endocrine Society on Friday afternoon, June 20, will be on "Diabetes and Metabolism," and a panel discussion entitled "Clinical Treatment of Diabetes" will be presented on Saturday, June 21, from 3:30 p.m. to 5:00 p.m.

The printed program of the American Diabetes Association, containing abstracts of papers to be presented, will be available at the registration desk at $1.00 each. All members will be sent a free copy in advance of the meeting; however, the aforementioned charge will be made for additional copies secured at the time of registration.

The program of the Eighteenth Annual Meeting follows, including pertinent information about the Scientific Sessions:

Registration: Friday, June 20, 2:00 p.m.—7:00 p.m.
Saturday, June 21, 8:30 a.m.—5:00 p.m.
Sunday, June 22, 8:30 a.m.—5:30 p.m.

Social Hour: Saturday, June 21, 6:30 p.m.

Banquet: Saturday, June 21, 7:30 p.m.

Annual Business Meeting: Sunday, June 22, 2:00 p.m.

SCIENTIFIC SESSIONS

Saturday, June 21, 9:00 a.m.

JOHN A. REED, Presiding

1. **Hyperglycemic Effects of Glucocorticoids in Man**
   KELLY M. WEST, University of Oklahoma School of Medicine and the Veterans Administration Hospital, Oklahoma City, Okla.

2. **Comparative Metabolism of Sorbitol and Fructose in Normal and Diabetic Subjects**
   ALBERT E. RENOLD, JEAN-PIERRE FELBER (by invitation), BURIS BOSHELL, DONALD B. MARTIN (by invitation), and GEORGE W. THORN, Baker Clinic Research Laboratory, Harvard Medical School, Boston, Mass.

3. **Specific Diabetic Vascular Lesions of the Lower Extremity: A Clinical and Pathological Correlation**
   SIDNEY GOLDENBERG, MORRIS ALEX (by invitation), and HERMAN T. BLUMENTHAL (by invitation), The Jewish Hospital and St. Louis University and Washington University Schools of Medicine, St. Louis, Mo.

4. **Electron Microscopy of the Effects of Glucagon on the Pancreas of Rats**
   PAUL E. LACY, A. F. CARDEZA (by invitation), and WILLIAM D. WILSON (by invitation), Washington University School of Medicine, St. Louis, Mo.

5. **Pancreatic Adaptation to Diabetogenic Hormones**
   SYDNEY S. LAZARUS and BRUNO W. VOLK (by invitation), Isaac Albert Research Institute, Jewish Chronic Disease Hospital and Albert Einstein Medical College, Brooklyn, N.Y.

BANTING MEMORIAL LECTURE

The Prediabetic State in Man: Definition, Interpretation and Implications

JEROME W. CONN, Professor of Medicine and Director of the Metabolism and Research Unit, University of Michigan Medical School; Chief, Department of Endocrinology and Metabolism, University Hospital, Ann Arbor, Mich.

6. **Insulin-like Activity of Normal and Diabetic Human Serum**
   PAUL M. BEIGELMAN, University of Southern California School of Medicine and Los Angeles County General Hospital, Los Angeles, Calif.

7. **The Use of Rat Adipose Tissue for the Measurement of Insulin-like Activity**
   DONALD B. MARTIN (by invitation), ALBERT E. RENOLD and YVES M. DAGENAIS (by invitation), Baker Clinic Research Laboratory, Harvard Medical School, Boston, Mass.

   Discussion of papers 6 and 7 opened by FRANCIS D. W. LUKENS, George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia, Pa.

8. **Structure and Function in Diabetic Nephropathy: The Importance of Diffuse Glomerulosclerosis**
   DEREK D. GELLMAN (by invitation), CONRAD L. PIRANI (by invitation), JOHN F. SOOTHILL (by invitation), ROBERT C. MUEHRCKE (by invitation), WILLIAM MADUROS (by invitation), and ROBERT M. KARK (by invitation), Presbyterian—St. Luke's, Cook County and the Research and Educational Hospitals, and the University of Illinois College of Medicine, Chicago, Ill.

Sunday, June 22, 9:00 a.m.

JOHN A. REED, Presiding

9. **The Effect of Glucose and Insulin on Amino-Acid Incorporation into Muscle Protein**
   IRA G. WOOL and M. E. KRAHL, University of Chicago, Chicago, Ill.

10. **Extrahepatic Oxidation of Palmitic Acid in the Diabetic Rat: Its Regulation by Insulin**
    W. J. LOSSOW (by invitation), R. HILL (by invitation), and I. L. CHAIKOFF, University of California School of Medicine, Berkeley, Calif.

11. **Studies of Abnormal Fat Metabolism in Patients with Diabetes Mellitus**
    THOMAS N. ROBERTS (by invitation), The New York Hospital, New York, N.Y.

12. **Relationship between Dietary Fat Content and Blood Lipids in Two Well Controlled Diabetic Subjects**
    F. C. GOETZ, GEORGE SCHROEPFER, JR., (by invitation), and BRADFORD FRIEDRICH (by invitation), University of Minnesota Medical School, Minneapolis, Minn.

13. **Use of Special Diets in Diabetic and Nondiabetic Patients with Peripheral Vascular Disease and in Diabetic Patients with Retinal and Renal Disease**
    L. W. KINSELL, G. D. MICHAELS (by invitation), S. SPLITTER (by invitation), and P. WHEELER (by invitation), Institute for Metabolic Research, Highland Alameda County Hospital, Oakland, Calif.

    Discussion on Fat Metabolism (papers 9-13) opened by HENRY T. RICKETTS, University of Chicago, Chicago, Ill.

14. **The Effect of Prior Carbohydrate Intake on the Oral Glucose Tolerance Test**
    HUGH L. C. WILKERSON, U. S. Public Health Service, Boston, Mass.

15. **Clinical and Laboratory Studies on Insulin Resistance**
    DACE B. MITCHELL (by invitation), GEROLD M. GRODSKY (by invitation), TOSHIO TORII (by invitation), VINCENT C. Di RAIMONDO (by invitation), and PETER H. FORSHAM, University of California School of Medicine, San Francisco, Calif.

16. **Observations Concerning a Humoral Insulin Antagonist During Diabetic Ketosis**
    JAMES B. FIELD, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

Sunday, June 22, 2:30 p.m.

ALEXANDER MARBLE, Presiding

17. **Effects of 2-Deoxyglucose on Glucose Metabolism in the Rat and on the Hypoglycemic Response to Tolbutamide**
    JOSIAH BROWN (by invitation), and HOWARD L. BACHRACH (by invitation), University of California School of Medicine at Los Angeles, Calif.

18. **The Role of the Anterior Pituitary in the Response of Animals to Tolbutamide**
W. E. DULIN (by invitation), and W. L. MILLER, JR., (by invitation), Research Laboratories, The Upjohn Company, Kalamazoo, Mich.

19. **Effects of Insulin and Tolbutamide on the Turnover Rate of Blood Glucose in Humans**
A. GERSON JACOBS (by invitation), GEORGE A. REICHARD (by invitation), EDWARD H. GOODMAN, JR., (by invitation), BERNICE FRIEDMANN (by invitation), and SIDNEY WEINHOUSE, The Albert Einstein Medical Center and the Institute for Cancer Research, Fox Chase, Philadelphia, Pa.

20. **Long-term Clinical and Laboratory Studies in Diabetic Patients Treated with Tolbutamide**
WILLIAM W. H. POTE, JR., ELMER A. ANDERSON (by invitation), and BURT COCHRAN, JR., (by invitation), College of Medical Evangelists, Los Angeles, Calif.

21. **Pharmacology of N-(p-Chlorophenylsulfonyl)-N'-n-propylurea (Chlorpropamide)**
MARY A. ROOT, The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.

22. **Clinical Use of the Biguanides and Their Role in Stabilizing Juvenile-Type Diabetes**
LEO P. KRALL and PRISCILLA WHITE, Joslin Clinic, Boston, Mass.

Discussion of Oral Hypoglycemic Compounds (papers 17-22) opened by ALEXANDER MARBLE, Harvard Medical School, Joslin Clinic and New England Deaconess Hospital, Boston, Mass.

BY TITLE

Diabetes of Twenty or More Years' Duration Studied in a Large County Hospital
RICHARD E. BAILEY (by invitation), BURT COCHRAN, JR., (by invitation), and EUGENE BERMAN, University of Southern California School of Medicine, College of Medical Evangelists, and the Los Angeles County Hospital, Los Angeles, Calif.

Antibiotic Patterns of Organisms Recovered from Diabetic and Nondiabetic Urine
EUGENE E. BERMAN and MARJORIE BIDDLE (by invitation), Los Angeles County Hospital and University of Southern California School of Medicine, Los Angeles, Calif.

Effects of Estrogen on Diabetic and/or Hyperlipemic Humans
EDWIN BOYLE, JR., Medical College Hospital, Charleston, S. C.

Hypophysectomy for Diabetic Sequelae
JOHN B. BRYAN, W. L. LOWRIE, W. E. REDFERN and FRED W. WHITEHOUSE, Henry Ford Hospital, Detroit, Mich.

The Effect of Denervation on the Insulin Sensitivity of the Rat Diaphragm
MARIA GORDON BUSE (by invitation), JOHN F. BUSE (by invitation), and OLLIE SMITHWICK (by invitation), Medical College of South Carolina, Charleston, S. C.

Acute Effects of Insulin on Lipemia and Ketonemia of Pancreatectomized Rats
SIDNEY S. CHERNICK (by invitation), and ROBERT O. SCOW (by invitation), National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

Effect of Exercise on Glucose Utilization of Young Normal and Diabetic Subjects
JEAN CHRISTOPHE (by invitation), and JEAN MAYER (by invitation), Harvard School of Public Health, Boston, Mass.

Control of Pancreatic Endocrine Function by Glucose
ARTHUR R. COLWELL, JR., and ROBERT METZ (by invitation), Northwestern University Medical School, Chicago, Ill.

The Influence of Phenethylbiguanide (DBI) on the Metabolism of Lactic Acid in Diabetic Patients
JAMES W. CRAIG, MAX MILLER and HIRAM WOODWARD, JR., (by invitation), Western Reserve University School of Medicine, Cleveland, Ohio

Metabolic Effects of Phenethylformamidinyliminourea (DBI) in Normal Subjects and in Diabetic Patients
STEFAN S. FAJANS, JOHN A. MOORHOUSE, H. DOORENBOS (by invitation), LAWRENCE H. LOUIS (by invitation), and JEROME W. CONN, University of Michigan Medical School, Ann Arbor, Mich.

An Immuno-Chemical Method for the Determination of the Serum Level of Insulin
RICHARD J. FEINBERG (by invitation), University of Pennsylvania School of Medicine, Philadelphia, Pa., and University of Illinois Research and Educational Hospital, Chicago, Ill.

Mechanism of the Glucosuria Produced by Repeated Injections of Glucagon in Normal Subjects
JEAN-PIERRE FELBER (by invitation), and THEODORE B. VAN ITALLIE, Harvard School of Public Health, Harvard Medical School and the Peter Bent Brigham Hospital, Boston, Mass.

The Late Puerperal Glucose Tolerance of Mothers of Large Infants
PALMER H. FUTCHER, ALLAN L. HASLUP (by invitation), and BROCK O. NUGENT (by invitation), The Johns Hopkins University School of Medicine and Hospital, Baltimore, Md.

Maternal Prediabetes as a Cause of the "Unexplained" Stillbirth
W. P. U. JACKSON (by invitation), University of Cape Town, South Africa.

Clinical Observations with Phenethylbiguanide (DBI)
THOMAS H. LAMBERT, Scripps Clinic and Research Foundation, La Jolla, Calif.

Nineteen Months' Clinical Experience with a New Hypoglycemic Drug
JULIUS POMERANZE, New York Medical College—Metropolitan Medical Center, and Bird S. Coler Memorial Hospital, New York, N. Y.

Prediagnosis Diabetes
JULIUS POMERANZE, New York Medical College—Metropolitan Medical Center, and Bird S. Coler Memorial Hospital, New York, N. Y.

Turnover and Oxidation of Plasma Glucose in Humans as Influenced by Insulin and Tolbutamide
G. L. SEARLE (by invitation), G. E.

MORTIMORE (by invitation), R. BUCKLEY (by invitation), and W. A. REILLY (by invitation), Veterans Administration Hospital, San Francisco, Calif.

Neuropathic (Charcot) Joints Occurring in Diabetes Mellitus

WILLIAM M. SHEPPE, The Wheeling Clinic, Wheeling, W. Va.

The Effect of Insulin on Serum Lipoprotein Concentrations in Diabetic Patients

E. H. STRISOWER (by invitation), R. WEED (by invitation), J. W. GOFMAN (by invitation), B. STRISOWER (by invitation), and O. de LALLA (by invitation), University of California, Berkeley, Calif.

The Intravenous Tolbutamide Response Test as a New Test in the Diagnosis of Mild Diabetes: Evaluation in 242 Subjects

ROGER H. UNGER and LEONARD L. MADISON, University of Texas Southwestern Medical School and the Veterans Administration Hospital, Dallas, Tex.

Diabetes in Industry

LEO WADE, Esso Standard Oil Company, New York, N. Y.

Preliminary Clinical Observation on the Use of N-B-Phenethylformamidinyliminourea (DBI) as an Oral Hypoglycemic Substance

CHARLES WELLER and ALICE MACAULAY (by invitation), Grasslands Hospital, Valhalla, N. Y.

Depression of Glucagon Induced Hyperglycemia by 17-Ethyl-19 Nor-Testosterone

SHIRLEY WEISENFELD and MARTIN G. GOLDNER, Jewish Chronic Disease Hospital, Brooklyn, N. Y.

The Effect of 6-Deoxy-6-fluoroglucose on the Metabolism of Glucose

ARNE N. WICK (by invitation), GEORGE S. SERIF (by invitation), ELLEN LARSON (by invitation), and CHARLES J. STEWART (by invitation), Scripps Clinic and Research Foundation and San Diego State College, La Jolla and San Diego, Calif.

ADA RESEARCH FELLOWSHIP

The Committee on Research and Fellowships of the American Diabetes Association announces that it will award at least one Fellowship for the academic year 1959-60. The deadline for applications is Nov. 15, 1958. Requests for application forms and other inquiries should be addressed to Mr. J. Richard Connelly, Executive Director, who will forward the information to the Committee.

NEW MEMBERS

Active

The following were elected as of April 1, 1958, and May 1, 1958:

California	
Munson, Ralph E.	Menlo Park
Colorado	
Elrick, Harold	Denver
Florida	
Abelove, William A.	Miami
Collins, Grover C.	Palatka
Schneider, Irvin C.	Jacksonville
Terragni, Manlio	Miami
Warren, Lyman O.	St. Petersburg
Werblow, Sol C.	Miami Beach
Georgia	
Bramblett, Rupert H.	Cumming
Clark, Sarah L.	Augusta
Davidson, Charles W.	Decatur
Kane, Eugene C.	St. Simons Island
Parks, Harry	Atlanta
Raiford, Morgan B.	Atlanta
Victor, Jules, Jr.	Savannah
Wilmer, J. Grant	Atlanta
Illinois	
Chertack, Melvin M.	Chicago
Indiana	
Hinshaw, Horace D.	La Porte
Rubright, Robert L.	Hammond
Kentucky	
Tillett, Robert S.	Louisville
Maine	
Storer, Daniel P.	Portland
Maryland	
D'Antonio, Joseph	Baltimore
Kress, Milton B.	Baltimore
Massachusetts	
Hadley, William B.	Boston
Michigan	
Bryan, John B.	Detroit
Wilson, Doyle E.	Kalamazoo
Mississippi	
Barnes, George S.	Columbus
Missouri	
Levin, Marvin E.	St. Louis
Vacca, Joseph B.	St. Louis
New York	
Clinton, Marshall	Buffalo
Dumm, Mary E.	New York
Martus, Grace R.	Rockaway Beach
Montgomery, Warren R.	Buffalo
Van Itallie, Theodore B.	New York
North Carolina	
Gridley, Timothy H.	Fayetteville
Merritt, Jesse F.	Greensboro
Saravia, Alfredo	Durham
Verner, John V., Jr.	Durham
Whitfield, Bryan W.	Murphy
Yount, Ernest H., Jr.	Winston-Salem
Ohio	
Schuster, Fred J.	Cleveland
Pennsylvania	
Nevin, Robert J.	Washington
Stockdale, Robert H.	Tarentum

South Carolina	
McLain, William C., Jr.	Columbia
Warren, Allan B., Jr.	Spartanburg
Tennessee	
Roehm, Dan C.	Nashville
Vermont	
Sims, Ethan A. H.	Burlington
Wisconsin	
Danford, Harold G.	Appleton
McMahon, F. Gilbert	Madison
Other Countries	
Canada	
Campbell, James	Toronto
Ziegler, Leopold M.	Montreal
Japan	
Yoshida, Tsuneo	Osaka

NEWS OF AFFILIATE ASSOCIATIONS

The DIABETES ASSOCIATION OF GREATER CLEVELAND (Professional Section) will hold its annual Dinner Meeting May 23 at the Wade Park Manor in Cleveland. Solomon A. Berson, M.D., Chief Radio-Isotope Service, Veterans Administration Hospital, Bronx, New York, will be guest speaker talking on "Insulin-binding Antibodies and Insulin Resistance."

The WASHINGTON DIABETES ASSOCIATION in cooperation with the University of Washington School of Medicine sponsored its Fourth Annual Symposium on Metabolic and Endocrine Diseases April 19 in Seattle.

The morning program included the following: "Introduction," by O. C. Olson, M.D.; "Pancreatic Mechanisms in Diabetes," Arnold Lazarow, M.D., Ph.D., Minneapolis; "Symposium on Certain Aspects of Calcium Metabolism," moderated by Robert H. Williams, M.D., including "Pathology," by Gordon D. LaZerte, M.D.; "Homeostasis and Hormonal Regulation," by Gilbert S. Gordan, M.D., University of California; "Gastrointestinal Absorption," by Wade Volwiler, M.D.; "Panel —with Case Presentations (1. Hyperparathydroidism 2. Milk Alkali Syndrome)," by Dr. Williams, Dr. Gordan, Robert L. Nielsen, M.D., and Elizabeth Knapp Smith, M.D.

During the afternoon sessions, the following program was presented with Joseph H. Crampton, M.D., as moderator: "Metabolic Bone Disease," by Dr. Gordan; "Metabolic Defects in Diabetes in Relation to Symptoms," by Dr. Lazarow. A panel was also presented with John Hogness, M.D., as moderator. Entitled "Sex: What Is It?," participants included Thomas Shepard, M.D., Gilbert Greenwald, M.D., and Dr. Gordan.

NEWS NOTES

PERSONALS

CHARLES H. BEST, M.D., co-discoverer of insulin, has recently been made an Honorary Life Member of the Venezuelan Antidiabetic Association, First Honorary Life Member of the Venezuelan Endocrine Society, and Member of the Venezuelan Society for the Advancement of Science. Another high honor was granted him in Venezuela recently when he received the Freedom of the City of Caracas from the Council of that City.

In 1957 Dr. Best was unanimously elected by the Board of Directors of the American Physicians Art Association to be the first Honorary President of that Association.

HELEN E. MARTIN, M.D., Los Angeles, will present the following papers at the University of Southern California School of Medicine Postgraduate Refresher Course to be held in Hawaii and on board the S. S. Matsonia Aug. 5-21, 1958: "Drug Therapy of Hypertension"; "Cushing's Syndrome"; "Peripheral Vascular Disease in the Diabetic, New Approaches to Therapy"; "Workshop in Electrolytes"; "Office Management of Diabetes"; "Magnesium Metabolism"; "Management of Diabetes, Pre- and Postoperatively"; "The Diabetic in Pregnancy"; "Pyelonephritis."

OBITUARY

PATRICK JAMES HAND, M.D., Glenolden, Pennsylvania, was born March 24, 1909, in Shenandoah, Pennsylvania, and died at the age of forty-eight. Dr. Hand, a member of the American Diabetes Association since 1949, was Chief of Medicine at Fitzgerald-Mercy Hospital, Darby, Pennsylvania. A graduate of the University of Pennsylvania Medical School in 1935, he served as a Lieutenant Colonel in the U. S. Army during World War II.